Dissertation on

DOPPLER ULTRASOUND ASSESSMENT OF TUMOUR VASCULARITY IN LOCALLY ADVANCED BREAST CANCER AT DIAGNOSIS AND FOLLOWING PRIMARY SYSTEMIC CHEMOTHERAPY

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CERTIFICATE

This is to certify that **Dr.R.S.AARATHHI DHEVI** has been a post graduate student during the period May 2010 to April 2013 at Barnard Institute of Radiology, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai.

This Dissertation titled **"DOPPLER ULTRASOUND ASSESSMENT OF TUMOUR VASCULARITY IN LOCALLY ADVANCED BREAST CANCER AT DIAGNOSIS AND FOLLOWING PRIMARY SYSTEMIC CHEMOTHERAPY"** is a bonafide work done by her during the study period and is being submitted to the Tamilnadu Dr. M.G.R. Medical University in partial fulfillment of the M.D. Branch VIII Radiodiagnosis Examination

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DECLARATION

I **Dr.R.S.AARATHHI DHEVI** solemnly declare that this dissertation entitled, **"DOPPLER ULTRASOUND ASSESSMENT OF TUMOUR VASCULARITY IN LOCALLY ADVANCED BREAST CANCER AT DIAGNOSIS AND FOLLOWING PRIMARY SYSTEMIC CHEMOTHERAPY"** is a bonafide work done by me at the Barnard Institute of Radiology, Madras Medical College and Rajiv Gandhi Government General Hospital during the period 2010 – 2013 under the guidance and supervision of the Director, Barnard Institute of Radiology of Madras Medical College and Government General Hospital, Professor **Dr. K. Vanitha, M.D., D.M.R.D., D.R.M.,** This dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree Radiodiagnosis.**

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CONTENTS

ABSTRACT

TITLE: Doppler ultrasound in assessment of vascular response to primary systemic chemotherapy in locally advanced breast cancer.

OBJECTIVE. To assess the response of tumor vascularity to primary systemic chemotherapy using doppler in patients with locally advanced breast cancer, and to compare it with morphological response by clinical and sonographic assessment with histopathology as gold standard.

MATERIAL AND METHODS: 54 patients with non-metastatic locally advanced breast cancer underwent detailed clinical examination, ultrasonography of breast and axillae, and color and spectral doppler examination at baseline and before every cycle of chemotherapy (FAC regimen) to assess response to chemotherapy. Doppler indices(PSV, EDV, RI and PI) and tumor vascularity(no. of colour signals) were recorded prior to every cycle. Assessment for operability was done at end of 4 cycles and patients were referred to surgical department for curative surgery. The results of clinical examination, ultrasonography and color doppler were compared with results obtained from histopathology.

RESULTS: Doppler was able to predict the response to chemotherapy earlier in 90% of cases. The best predictor for complete pathological response was found to be disappearance of all vascular signals within the tumor. A serial increase in the RI of tumor vessels was found to very good in predicting complete pathological response with a sensitivity, specificity, PPV and NPV of 80%, 93.4%, 66.7% and 95.6% respectively. There was no significant difference in doppler angle dependent indices – PSV & EDV- between responders and non-responders

CONCLUSION: Colour Doppler is a cost-effective method to assess response to chemotherapy in patients with locally advanced breast cancer.

KEYWORDS : Locally advanced breast cancer, primary systemic chemotherapy, Doppler ultrasound, Resistivity Index.

INTRODUCTION

Breast cancer is the most commonly occurring cancer in women and the most frequent cause of female cancer death in the world as a whole .Overall, in India, its incidence ranks second, next to that of cervical cancer. But recently it has been found that it is the most common cancer in women in the metropolitan cities.[1,2] The incidence rates are increasing and a higher proportion of newly diagnosed cases are in late stage of disease resulting in an increase in morbidity.

Among those who present with locally advanced disease, the subset of patients who have metastatic disease can only be offered palliative therapy and their 5 year survival rate is dismal (20%).[3] Those who have locally advanced disease but no evidence of systemic metastases, can be treated with an intent to cure. Since in our country 30- 60% of patients present with advanced stage and among them almost half have non metastatic locally advanced disease, disease mortality can be greatly reduced by proper management of these patients.

The treatment options for locally advanced breast cancer include surgery followed by adjuvant chemotherapy and primary systemic chemotherapy (earlier known as neo adjuvant chemotherapy) followed by surgery with or without adjuvant chemotherapy and radiotherapy. Neoadjuvant chemotherapy is the norm now because it can downstage the tumour and convert inoperable tumours into operable ones thereby avoiding morbid, mutilating surgeries.

It has been proven that pathological complete response to primary systemic chemotherapy is a powerful predictor of prolonged disease free survival in patients with locally advanced breast cancer. But response to neoadjuvant chemotherapy is highly variable and ranges from 30-100%. Among patients with locally advanced breast cancer (LABC) around 3-20% do not respond to chemotherapy according to literature.

If the response to chemotherapy can be predicted earlier during the course of the chemotherapy, we would be able to stop further administration of the chemotherapy regimen to people who are not responding, thereby saving these patients from the toxicity of the inefficacious chemotherapeutic agents. As of now, in India, clinical examination is the most widely used method to assess response to chemotherapy. Clinical examination and ultrasonography use morphological criteria, mainly change in size, to assess tumour response to treatment.

Tumour vascularity has received much attention of late and it has been postulated that neoangiogenesis in tumours is an important phenomenon essential for the sustenance and proliferation of cancer cells. It has also been suggested that the tumour vessels may respond to chemotherapeutic agents even before there is significant reduction in tumour size.

Assessment of tumour vascularity can be done by Doppler ultrasound and dynamic contrast enhanced MRI. Studies on MRI done in the western world have revealed MRI has a very good accuracy in detecting response. But the expense involved in doing MRI is prohibitive when considered for application to a large number of people especially in a low income country like India. Doppler ultrasound is non invasive and less expensive and is therefore a more practical option in a developing country like ours. This prospective study was done with objective of ascertaining the usefulness of Doppler in predicting the response of LABC to neoadjuvant chemotherapy

AIMS AND OBJECTIVES

1. To evaluate Doppler ultrasound parameters in assessment of vascular response to chemotherapy in patients with locally advanced breast cancer and to compare it with morphological response by clinical examination and ultrasound taking post-surgical histopathological response as gold standard
2. To ascertain the ability of Doppler ultrasound to predict the response early during the course of chemotherapy.

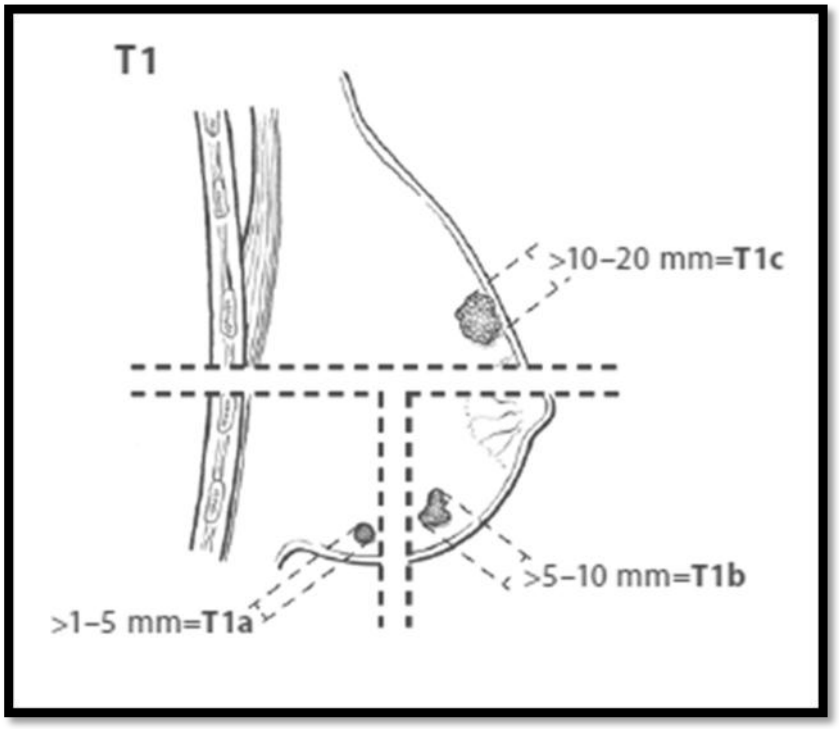
REVIEW OF LITERATURE

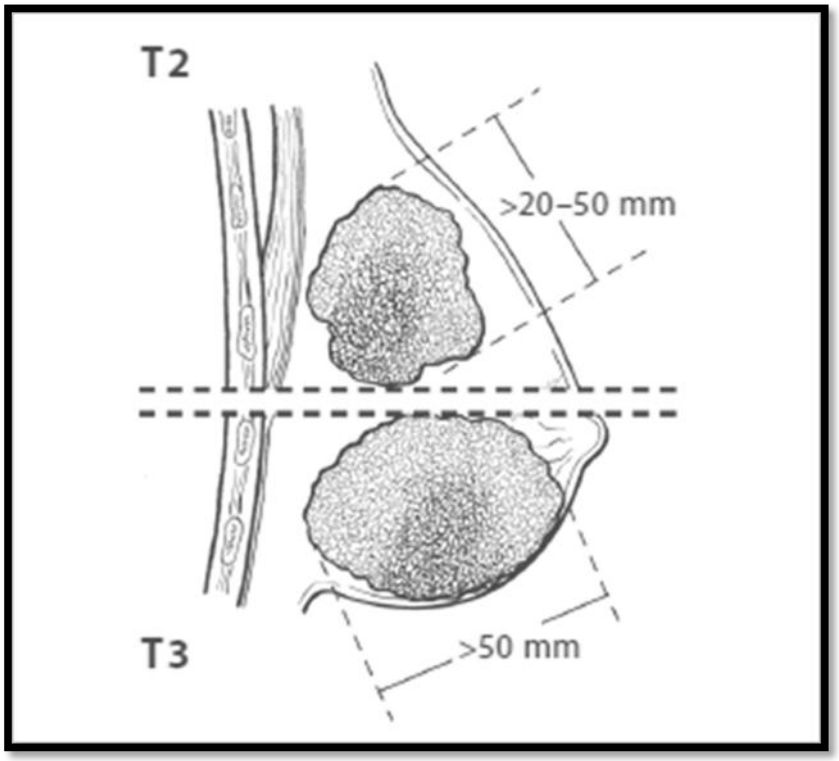
Breast cancer is on the rise worldwide including the developing world. It is a major cause of female cancer mortality. Since there is no structured screening programme to detect breast cancer in India and since the awareness among women is very low, breast cancer is not detected early in a vast majority of cases. So, the incidence of advanced breast cancer which includes non-metastatic locally advanced and metastatic disease is much higher in developing countries than the western world.

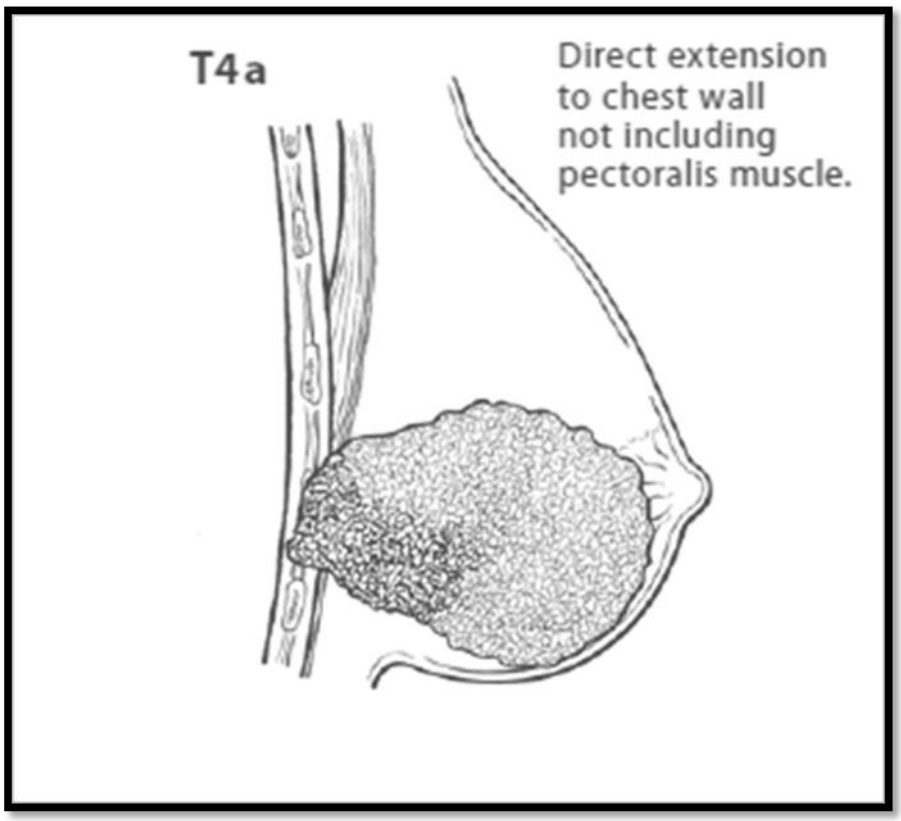
The 7th edition of AJCC cancer staging manual stages breast cancer as follows.[4,5]

BREAST CANCER STAGING – AJCC MANUAL 7th EDITION

T1	Tumor ≤ 20 mm in greatest dimension
T1mi	Tumor ≤ 1 mm in greatest dimension
T1a	Tumor > 1 mm but ≤ 5 mm in greatest dimension
T1b	Tumor > 5 mm but ≤ 10 mm in greatest dimension
T1c	Tumor > 10 mm but ≤ 20 mm in greatest dimension
T2	Tumor > 20 mm but ≤ 50 mm in greatest dimension
T3	Tumor > 50 mm in greatest dimension
T4	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules) Note: Invasion of the dermis alone does not qualify as T4
T4a	Extension to the chest wall, not including only pectoralis muscle adherence/invasion
T4b	Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d'orange) of the skin, which do not meet the criteria for inflammatory carcinoma
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma


T1
>10–20 mm=T1c
>5–10 mm=T1b
>1–5 mm=T1a


T2
>20–50 mm
T3
>50 mm



Regional Lymph Nodes (N)

CLINICAL

NX	Regional lymph nodes cannot be assessed (for example, previously removed)
N0	No regional lymph node metastases
N1	Metastases to movable ipsilateral level I, II axillary lymph node(s)
N2	Metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted; or in clinically detected* ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastases
N2a	Metastases in ipsilateral level I, II axillary lymph nodes fixed to one another (matted) or to other structures
N2b	Metastases only in clinically detected* ipsilateral internal mammary nodes and in the absence of clinically evident level I, II axillary lymph node metastases
N3	Metastases in ipsilateral infraclavicular (level III axillary) lymph node(s) with or without level I, II axillary lymph node involvement; or in clinically detected* ipsilateral internal mammary lymph node(s) with clinically evident level I, II axillary lymph node metastases; or metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
N3a	Metastases in ipsilateral infraclavicular lymph node(s)
N3b	Metastases in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
N3c	Metastases in ipsilateral supraclavicular lymph node(s)

Distant Metastases (M)

- **M0** No clinical or radiographic evidence of distant metastases
- **cM0(i+)** No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other nonregional nodal tissue that are no larger than 0.2 mm in a patient without symptoms or signs of metastases
- **M1** Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven larger than 0.2 mm

ANATOMIC STAGE/PROGNOSTIC GROUPS			
Stage 0	Tis	N0	M0
Stage IA	T1*	N0	M0
Stage IB	T0	N1mi	M0
	T1*	N1mi	M0
Stage IIA	T0	N1**	M0
	T1*	N1**	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2	M0
	T1*	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

Locally advanced breast cancer essentially includes all stage IIIB and IIIC tumours along with a small subset of stage IIIA tumours. It is a heterogeneous entity including cases which have advanced disease either in

a. primary tumour (T3 - tumours >5cm,
T4 - skin involvement, chest wall involvement and inflammatory tumours)

b. nodal stage (fixed ipsilateral axillary nodes,
ipsilateral internal mammary nodes,
ipsilateral infraclavicular nodes,
ipsilateral supraclavicular nodes)

c. Both

All of these would fall under stage III except T3 N0 tumours which fall under stage IIB. These tumours are further classified as those that are operable and those that are inoperable.[6]

- Operable LABC – Stage IIB and IIIA (T3 and N2 tumours)
- Inoperable LABC – Stage IIIB and IIIC (T4 and N3 tumours)

Most invasive breast cancers are considered to be systemic diseases at diagnosis in lieu of micrometatases to distant sites. According to the guidelines published by National Collaborating Centre For Cancer at Wales, chemotherapy to eradicate micrometastases is indicated even in patients with early breast cancer based on the prognostic factors of the individual tumour.[7] So, locally

advanced tumours require multimodality treatment for adequate disease control.[8,9,10,11]

The operable tumours have the option of undergoing curative surgery followed by adjuvant chemotherapy. Neoadjuvant chemotherapy can also be used in such cases so as to reduce the tumour size, offer breast conservation and avoid mutilating surgeries. Neoadjuvant chemotherapy has been found to be atleast as effective as adjuvant therapy in the NSABP B-18 study, making either approach reasonable for a woman with operable breast cancer. The survival rates of women treated with adjuvant or neoadjuvant chemotherapy are equivalent.[12]

But in case of tumours that are inoperable at presentation, the option of primary surgery does not exist. Also, there is increased possibility of micrometastases. So, neoadjuvant chemotherapy followed by surgery with or without adjuvant chemotherapy and radiotherapy is the current norm for such patients.[8] Neoadjuvant chemotherapy is also very effective in downstaging the tumours rendering inoperable tumours operable.[9,10]

Primary systemic chemotherapy also has the advantages of delivery of drugs through intact vasculature and the opportunity to study the biologic effects of chemotherapy in vivo. Disadvantages are the resultant loss of important pathologic prognostic markers such as initial tumour size and the number of axillary lymph nodes involved.

There are several prognostic indices for breast cancer such as the histological grade, stage at presentation and receptor status. For tumours that have been treated with chemotherapy, response to chemotherapy is a powerful predictor of disease free survival and it is the most commonly used one.[13] In a study by Feldman *et al*, patients without gross residual tumour after chemotherapy had a survival rate at 6 years of 93% whereas those with residual tumour had a survival rate of only 34%.[14] In a study by Chollet et al which included 451 patients, the difference in disease free survival among those patients who had achieved pathological complete response and those who had not was statistically significant with relapse rates being 18.3% in patients who attained pCR and 35.4% in patients who did not.[15]

Apart from the prognostic value, prediction of response to chemotherapy early during the chemotherapeutic regimen is of great practical value in the management of individual patients. If non responders are identified early, the remainder of the inefficacious regimen can be terminated, thereby protecting the patients from their unwanted toxic effects and alternative treatment strategies can be offered which could help in disease control in such patients. Such strategies include switching over to an alternative regimen of non-cross resistant chemotherapeutic drugs or early surgery.

The response to chemotherapy in individual tumours varies as breast cancer is a biologically heterogeneous entity. Needless to say the chemotherapeutic agent used also plays an important role in determining pathological response and thereby has a bearing on patient survival. The NSABP B-27 study and Aberdeen trial (TAX 301) reported better breast conservation rates and pathological complete response rates with addition of docetaxel to the chemotherapeutic regimen.[16,17]

Thus, assessment of response to chemotherapy plays an important role both in the management and prognostication of patients with breast cancer. Various methods of assessment have been studied by researchers worldwide. They can be summarised as follows

1. Clinical assessment
2. Laboratory assessment
    - Histopathological markers like tumour grade, receptor status and expression of angiogenic markers like VEGF, CD-105.
    - Serum biomarkers like MIF, MMP-2, EGFR

3. Imaging
    - Morphological imaging: X Ray mammography, B mode ultrasound, Computed Tomography, Magnetic resonance imaging
    - Functional imaging:
        1. Vascular imaging: Doppler ultrasound, Doppler ultrasound with micro bubble contrast agent, Dynamic contrast enhanced MRI
        2. Non vascular imaging: Diffuse Optical Spectroscopy, Positron Emission Spectroscopy, Mammoscintigraphy.

CLINICAL EXAMINATION

Clinical assessment is by far the most widely used method for evaluation of response to chemotherapy in developing countries like ours. It is easily available, inexpensive and less time consuming. But it has been found to be inaccurate in many studies. Khokher et al have concluded that sensitivity of initial clinical response to predict the clinical response at the end of chemotherapy were high at 91% and 83% respectively with an accuracy of 83%. They also found that initial clinical progressive disease had very good sensitivity, specificity and accuracy for predicting clinically progressive disease at the end of chemotherapy. But the authors had not compared the correlation

between the initial or final clinical response with pathological response.[18] Other studies quoted later reveal that the correlation of clinical response with pathological response was neither consistently nor substantially significant.

In a series of 141 patients, Fiorentino et al have reported that clinical examination was better in the prediction of complete pathological response and that it correlated significantly with disease free survival. But the authors had compared clinical examination only with morphological imaging modalities – mammography and ultrasound, and not with the newer functional imaging modalities.[19]

Von Minckwitz et al have reported in their study that patients with a clinical complete or partial response were 3.3 times more likely to achieve a pCR than those who did not. But they have also concluded that though the clinical response after two cycles is a strong predictor it is not independent.[20]

In a study by Cocconi et al, it was found that clinical assessment had a false positive rate of 22.9% and false negative rate of 8.9% in assessing response to chemotherapy.[21] In a series of 141 patients, Sun et al found that the false complete remission rate as judged by clinical examination was 46.8%.[22]

Helvie et al have concluded that the sensitivity of clinical examination to identify residual tumor mass after neoadjuvant chemotherapy in 56 patients with LABC was only 49%.[23] Chagpar et al in a study with 189 patients found that the correlation between clinical and pathological response was poor (Kappa value between 0.24 and 0.35).[24] Similarly Yeh et al have found that the

agreement between clinical assessment and pathological assessment for response is only 19%.[25]

The disadvantage with clinical examination is that when sometimes the mass resolves on chemotherapy leaving behind viable tumour cells, it can become undetectable by palpation. Also the high rate of underestimation of response may be due the fact that palpation cannot differentiate between a fibrotic or necrotic mass and viable residual tumour mass. This seems to be a significant fact as most tumours respond to chemotherapy by a process of fibrosis and necrosis. Also, tumour cell exposure to chemotherapeutic drugs like anthracyclines has been shown to induce immunostimulatory apoptosis and presentation of damage-associated molecular patterns which in turn result in the production of pro inflammatory cytokines resulting in acute inflammation.[26,27] These chemotherapy induced inflammatory edematous changes (incited by the death of tumour cells) can simulate increase in tumour size on palpation.

MAMMOGRAPHY

Fiorentino et al concluded that mammography fared worse than clinical examination in predicting residual tumour size.[19] Chagpar et al showed that the accuracy of mammography to predict residual tumour size was 70% when compared to 75% by ultrasound and 66% by clinical examination. Sperber et al reported that mammography correlated with pathological response only in 50% of patients.[24]

Sun et al have stated that in 53.5% of patients, mammography could not identify the change in tumour size following chemotherapy when compared with pretreatment assessment and that in patients with partial response, there was no significant reduction in the range of microcalcification.[22]

The problem with mammography arises mainly in patients with dense breasts where the tumour mass cannot be defined sufficiently at baseline for future comparisons. Also even in patients with fatty breasts, tumours responding to chemotherapy predominantly by the processes of fibrosis or necrosis cannot be differentiated from non responding ones as the mammographic density remains the same. In a series by Keune et al, of the 144 tumours imaged by mammography, size was unable to be defined in 60 (41.7%).[28]

Huber et al, in their study, concluded that if the > 50% of the tumour margin can be defined at baseline by mammography, final mammographic tumour size correlated significantly with the pathological tumour size and suggested that for tumours whose margins are ill-defined at baseline, alternative imaging modalities should be considered for response assessment.[29]

ULTRASONOGAPHY

Definition of tumour margins are better by ultrasonography when compared with mammography except when there is multifocal of ill defined tumour. In a study by Keune et al, though ultrasound was able to size 91% of tumours initially, it could predict chemotherapeutic response only in 60%.[28] Balu Maestro et al, in a study of 60 tumours, found that ultrasonography could correctly predict residual tumour size only in 43% of tumours.[30] Other studies have found that ultrasound was not significantly better in than clinical examination or mammography.[19,31]

In multifocal and illdefined tumors the ability of ultrasound to delineate the difference between tumour tissue and normal tissue is less. Moreover when the size of the tumour exceeds the size of the probe which is the case in most of the patients with LABC, the accuracy of ultrasound in determining tumour size is, at best, modest. Fibrosis occurring in the course of response to chemotherapy can cause substantial post acoustic shadowing which in turn limits the ability of ultrasound to visualise residual tumour mass.

Many studies have reported the superiority of MRI in assessment of residual tumour size and multifocality.[32,33] This is possible due to the increased contrast resolution inherent to MR imaging.

The problem in morphological imaging modalities is that they image only the volume of the mass and not the viability of tumour cells. The relationship between tumour cells and stroma in an individual tumour is variable as breast cancer is very heterogeneous in its biology. Thus tumours that have responded well, with the tumour tissue replaced by fibrotic or necrotic mass, may be erratically classified as non-responding by morphological imaging. This may lead to deprivation of further chemotherapy to which the tumour is sensitive. On the other hand, viable tumour cells may remain inspite of resolution of the mass resulting in the impression of a responding tumour by morphological imaging. This may lead to continuation of ineffective chemotherapeutic regimen when an early regimen change or surgery could have helped the patient get adequate disease control thus resulting in loss of precious time.

This is where functional imaging is postulated to help. The metabolism of tumours has been found to be much more than that of normal tumour tissue. Menezes et al have found that breast cancer cells showed an over abundance of cytochrome oxidase, an oxidative mitochondrial enzyme, compared to the adjacent normal cells. They postulated that the cancer cells induce oxidative stress in adjacent normal fibroblasts by making them switch over to anaerobic glycolysis which results in the production of large amounts of lactate. This lactate is then used up by the cancer cells which retain their ability to metabolise lactate through their oxidative mitochondrial enzymes. This hypermetabolism

increases the need for vascularity.[34] So metabolism and vascularity can serve as markers for the viability of tumour cells. Functional imaging modalities give us a reliable idea about the viable tumour burden as they image or measure the vascular or metabolic indices.

Vascular imaging modalities aim at the assessment of changes in vascularity due to chemotherapy which in turn is dependent on the change in the metabolism of cancer cells. Tumour angiogenesis was the term coined by Folkman and Hannahan to refer to the induction of angiogenesis in a nascent tumour.[35]

During initial stages of tumour growth, nutrition is derived by diffusion of substrates along a concentration gradient. Tumours cannot grow beyond 2-3 mm unless vascularised as this is the distance limit for diffusion of substrates. When a tumour grows beyond this size, there is, inevitably, a zone of hypoxia in the centre of the tumour.[36] Hypoxia prevents degradation of Hypoxia inducible factor 1α (HIF-1α) which in turn activates the transcription of the genes of pro angiogenic proteins like Vascular Endothelial Growth factor (VEGF). These proteins are ultimately responsible for recruitment of new vessels – angiogenesis.[37] Thus there is an increase in mean vessel density in the tumour tissue compared to normal tissue. Thus, in locally advanced cancers of the

breast which are invariably large, angiogenesis definitely plays a major role in the sustenance of neoplastic cells.

But tumour vessels are abnormal intrinsically. They are dilated, tortuous and leaky (increased permeability). Their tunica media is not properly developed.[37] So the physiology and physics of blood flow through them are very different from normal vessels with flow in the tumour vessels being more turbulent and of a low resistance type.

Imaging of vascularity may be direct – imaging the vessels or its properties, or indirect - by imaging the uptake or distribution of metabolic products which are delivered to the tumour by the vessels, and thereby obtaining information about the vessels.

Direct vascular imaging modalities used at present are Doppler ultrasound with or without contrast agent and Dynamic contrast enhanced MRI.

DYNAMIC CONTRAST ENHANCED MRI

Contrast enhanced MRI employs 2 parameters – Intensity of enhancement that reflects the vessel density and rate of wash out which reflects the permeability of the tumour vessels. Though MRI does not image the tumour vessels directly, signal intensity in contrast enhanced images has been shown by Knopp et al to correlate with the vessel density in the

tumour.[38] Hulka et al suggested that the measurement of T1 value and extraction flow product correlated well with mean vessel density in the tumour.[39] Their findings were confirmed by Yeh et al.[40]

Many studies have measured the tumour size in the contrast enhanced images and compared it with size of the tumour in histopathology not taking into account the vascular characteristics (signal intensity or kinetics). Kawamura et al studied 11 patients with LABC on primary systemic chemotherapy with diffusion weighted and dynamic contrast enhanced MRI after each cycle of chemotherapy. They found that tumours that showed shrinkage in the area of contrast enhancement after the first cycle of chemotherapy showed pathological response. Though they used dynamic contrast enhanced MRI, they have not specified the phase in which the tumour size was measured.[41] Gilles and co-workers have reported that early contrast enhancement detected residual tumour in seventeen out of the eighteen patients.[42] Loo et al found that failure of the largest diameter of late contrast enhancement to decrease by more than 25% after two cycles of chemotherapy had a high specificity for predicting residual tumour at histopathology.[43] Abraham et al have reported an accuracy of 97% for MRI in predicting pathological residual disease.[44] Yeh et al found a 71% correlation between MRI and pathology in assessment of residual tumour,

which was far better than clinical examination mammography and ultrasound.[45] Similar results were obtained by Rosen et al.[46]

The main advantage in MRI is its ability to acquire dynamic images and compute time intensity curves which can help us image the increase in vascular permeability which is an important property of tumour vessels differentiating them from normal vessels.[37]

Knopp et al evaluated the vascular enhancement characteristics of breast tumours and compared it with tumour histology, expression of CD-31 (measure of vascular density) and VEGR (measure of vascular permeability). They have found significant correlation between tissue VEGF expression and vascular permeability to contrast agent as measured by the rate constant on MRI. They further suggested that in breast tumours responding to chemotherapy, decrease in vascular permeability as evidenced by the decrease in the rate of contrast enhancement, precedes the decrease in vascular density which is reflected by the decrease in signal intensity.[38,47] In contrast, studies by Ah see et al and Su et al have not found a statistically significant relationship between VEGF expression and vascular permeability characteristics on MRI.[48,49] El Khoury et al found that there was significant decrease in the washout volume of the tumour and Reiber et al found that tumours responding to chemotherapy

showed flattening of the time intensity curve after 2 cycles of chemotherapy.[50,51]

But changes in vascular permeability can be caused even by some chemotherapeutic drugs which could then become a confounding factor. Semb et al have found that docetaxel causes an increase in vascular permeability which leads to an initial increase in fluid filtration followed by plasma protein leakage.

So direct visualisation of vessels seems to be an easier and more convincing method of assessment of vascularity especially in follow up of proven cases of breast cancer on therapy. This can be done by doppler ultrasound.

INDIRECT IMAGING

MAMMOSCINTIGRAPHY

It uses technetium labelled MIBI (Methoxy Iso Butyl Isonitrile) to assess uptake by the tumour tissue. There are conflicting reports about the value of this modality. Mankoff et al in their study found that a decrease of 40% or more in the lesion to normal MIBI uptake ratio reliably predicted pathological complete response.[53] However, Travaini et al have claimed that pretreatment MIBI uptake and washout did not show any significant difference between responders

and non responders in their study of 51 patients.[54] The usefulness of scintimammography is questionable and the procedure involves exposure to ionising radiation as it entails the use of radioactive technetium.

POSITRON EMISSION TOMOGRAPHY

The uptake of glucose by the tumour has been found to correlate with the aggressiveness of the tumour. Mankoff et al have measured the tumour blood flow with ^{15}O – water and glucose metabolism with ^{18}FDG. They found that tumours that did not respond to NACT had significantly increased pre-treatment glucose metabolism.[55] In other studies there were conflicting results regarding the ability of PET to identify response to chemotherapy.[56,57,58,59]

Moreover the increased cost and reduced access to PET facilities prohibits its use in our country.

DIFFUSE OPTICAL SPECTROSCOPY

Diffuse optical spectroscopy (DOS) is now emerging as a non invasive indirect imaging modality of tumour metabolism. Pakalniskis et al studied the mean tumour haemoglobin levels with DOS and compared them with mean vessel density (MVD) of vessels expressing CD-105 and CD31 in the pre-treatment core biopsy specimen. MVD of CD105-expressing vessels correlated significantly with pre-treatment tumour haemoglobin levels in women who went on to have pCR and the MVD of CD31-expressing vessels correlated

significantly with pre-treatment tumour haemoglobin levels in women who went on to have pathological partial response (pPR). This indicates that the pre-treatment tumour haemoglobin reflects the vascularity of the tumour though the biomarkers expressed in the tumour vessels may vary.[60]

In a study by Soliman et al, 10 patients with LABC on PSC were followed up with diffuse optical spectroscopy at 1, 4 and 8 weeks after the commencement of chemotherapy. They found that percentage values of tumour oxygenated haemoglobin, water and scattering power were significantly different between responders and non responders as early as 4 weeks after starting chemotherapy.[61]

Roblyer et al studied 24 tumors under neoadjuvant chemotherapy with diffuse optical spectroscopy and found that there was a very significant difference in the tumour tissue oxyhemoglobin between responders and non responders and the difference was maximum just one day after starting chemotherapy.[62]

Cerussi et al showed in their study that Tissue optical index measurement of the tumours was able to differentiate between pCR and non –pCR tumours at the midpoint of therapy regardless of drug or dosing strategy. The difference was statistically significant.[63] In another study by the same authors which included 11 patients, they found that 1 week posttreatment tissue deoxyhemogobin concentration had 83% sensitivity and 100% specificity for

predicting therapeutic response while combined tissue deoxyhemogobin concentration and tissue water changes had 100% sensitivity and specificity

We see that different parameters were found significant in each of these studies. The technique of diffuse optical spectroscopy for purposes of medical imaging has not been standardised. In addition, special equipment is required for such studies along with optical compensation media whose accessibility and affordability are under question in a developing country like ours.

DOPPLER ULTRASOUND

Doppler ultrasound gives us an avenue to image the tumour vessels directly. The quantitative assessment is done by colour or power doppler. The assessment of flow characteristics in the tumoral vessels is done using spectral doppler.

Kedar et al concluded that the change in tumour vascularity as seen on colour doppler was able to predict the response to chemotherapy at least 4 weeks earlier than clinical examination and B mode ultrasound in 40% of patients.[65]

Huber et al have conducted a semiquantitative analysis of the detectable vessel density within the tumour tissue using colour Doppler. They have used a computer aided software to compute the colour pixel density (percentage of the pixels within the ROI occupied by colour) with a manually drawn ROI and

found that the agreement between the change in colour pixel density and histopathological response was substantial.[66]

Vallone et al have compared colour doppler and contrast enhanced colour doppler in identifying responders among 50 LABC patients on NAC. They have reported that in 9 cases conventional Doppler failed to detect tumor vascularity where as contrast enhanced doppler did. These cases were histologically proven to have residual disease. They concluded that contrast enhanced doppler was a more sensitive method to detect tumour vascularity and thereby residual tumour tissue.[67]

Kuo et al have quantified tumour vascularity using power doppler and computing vascularity index which is the ratio of number of pixels with colour to the total number of pixels within the ROI. They evaluated the patients on day 0, day 1 and day 8 of chemotherapy and found that vascularity index increased initially before decreasing when compared to the baseline. They found all patients with an initial increment in VI of >5% was found to respond to chemotherapy and that the change in VI predated the change in tumour size as assessed by clinical examination and B mode ultrasonography. Though they had used a software programme to calculate the vascularity index, they are of the opinion that a vascularity change of 5% could easily be detected by an experienced sonographer even without the assistance of computational software.[68]

There are two hypotheses regarding vascular resistance in tumour vessels. The presence of perfusion shortcuts due to the presence of arteriovenous shunts and the lack of vasomotor control due to the absence of muscle layer (tunica media) in the tumour vessels result in a low resistance flow. On the other hand increased vascular permeability results in leakage and accumulation of osmotically active substances in the interstitium giving rise to increased interstitial pressure which causes a high resistance to blood flow (viscous resistance). The counterbalancing effects both these factors could be expected to play a role in tumors and the so ultimate flow resistance has been found to be different in different areas within the same tumour.[70]

Kumar et al have studied 50 cases of locally advanced breast cancer with colour doppler twice during their treatment regime - at diagnosis and after three cycles of chemotherapy with CAF regimen. They put forth a colour doppler scoring system taking into account PSV, RI and PI to predict the pathological response giving each a score of 1,2,3 or 4 when there was a change of <25%, 25 – 50%, 50 – 75% or >75% from the baseline. They concluded that a cumulative Doppler score of >5 had a sensitivity of 91.7%. They also found that the complete disappearance of flow signals was highly specific for the prediction of complete responders.[71]

Singh et al have showed that tumours showing an increase in peak systolic velocity while on treatment had a greater likelihood of recurrence and that a decrease in peak systolic velocity correlated well with clinical response. The RI and PI did not show any consistent trend in their study.[72]

Osanai et al have found that lowest resistivity index of the tumour vessels shows significant correlation with tumour grade and Nottingham prognostic index. Nottingham prognostic index which is used to predict patient survival takes into account tumour size, nodal stage at presentation and histological grade.[73]

Several studies have shown that a higher RI was indicative of malignancy among breast masses. [74,75,76] In a series of cases, Oksuzoglu et al have reported that during the course of chemotherapy, the RI of the tumours that responded to chemotherapy decreased significantly (p value = 0.04). They also found that the decrease in RI was proportional to the decrease in size of the tumour.[77]

In a study on 49 cases of carcinoma of endometrium, Lee et al have found that RI values correlated significantly not only with histological grade and lymphovascular invasion but also with microvessel density and vascular endothelial growth factor (VEGF) levels.[78]

Greco et al studied the usefulness of colour doppler indices in 14 patients undergoing neoadjuvant chemotherapy for locally advanced carcinoma of the cervix. They reported a significant increase in RI and PI of both in the cervical artery and in the intratumoral vessels. The cervical arterial RI and PI were significantly less than that of healthy controls.[79]

In 2003, Alcazar et al conducted a study in 21 patients with locally advanced cervical cancer and found that the initial lowest RI, lowest PI and highest PSV of the intratumoral vessels had a significant correlation with final clinical response assessed at the end of concurrent chemoradiotherapy. As the patients did not undergo surgery, the results could not be compared with pathological response.[80]

Kerimoglu et al have compared colour Doppler ultrasound with dynamic contrast enhanced MRI for the prediction of response to radiotherapy to locally advanced cervical cancer. 13 cases underwent transvaginal ultrasound with colour and spectral Doppler examination 6 months before and 6 months after radiotherapy. 14 healthy controls also underwent transvaginal ultrasound with colour doppler for comparison. It was found that the RI of the cervical vessels was significantly lower in patients with cancer when compared to the controls. They found that in 10 out of the 11 patients who showed response to therapy, the post treatment RI showed a significant increase when compared with the

pretreatment RI. They also found a 100% correlation between MRI and RI for assessment of response to therapy.[81]

Jurado et al compared the lowest intratumoral PI measured by TVUS before surgery with the histological response. They found a significant association between the lowest PI and lymph node involvement, depth stromal invasion and lymphovascular invasion which are important predictors of survival. Using the cut off lowest PI <0.82 they were able to predict the need for further treatment after surgery.[82]

STUDY

AIMS AND OBJECTIVES

1. To evaluate Doppler ultrasound parameters in assessment of vascular response to chemotherapy in patients with locally advanced breast cancer and to compare it with morphological response by clinical examination and ultrasound taking post-surgical histopathological response as gold standard
2. To ascertain the ability of Doppler ultrasound to predict the response early during the course of chemotherapy.

MATERIAL AND METHODS

This was a prospective study conducted in madras medical college. Informed written consent was obtained from all patients prior to the start of the study. Institutional Ethics committee approval was obtained prior to the start of the study

INCLUSION CRITERIA

1. Female patients of 17 to 70 years of age with locally advanced breast cancer as per AJCC TNM staging (7^{th} edition)
2. Presence of histological proof of malignancy
3. Inoperability of the lesion at presentation
4. No clinical or radiological evidence of metastasis
5. No history of prior treatment for any malignancy
6. Fit for chemotherapy with anthracycline based regimen

    - ECOG performance status 0-2
    - Normal cardiac function (Echocardiographic ejection fraction >50%)
    - Normal renal and liver function tests
    - No other co morbid illness which precludes chemotherapy

EXCLUSION CRITERIA

1. Pregnant patients
2. Metastatic breast cancer
3. Patients unfit for chemotherapy
4. ECOG performance status >2
5. History of treatment for prior malignancy

METHODS:

All patients who fulfilled the inclusion criteria were subjected to a complete clinical examination of the breast, axilla and other major organ systems. Clinical examination of the breast entailed measurement of the breast mass in 2 perpendicular directions using vernier calipers, documentation of the surface, consistency, mobility, skin or chest wall invasion. The contralateral breast was also palpated for the presence of mass lesion. Both axillae were palpated for nodes and if present, their number, size and mobility were noted. Both supraclavicular fossae were palpated for the presence of nodes. Patients were then examined for clinical signs of metastases as evidenced by hepatomegaly, jaundice, ascites, pulmonary or neurological symptoms or signs and bone pain. The patients then underwent metastatic work up with ultrasonograms of the abdomen, CT scans of chest and abdomen and bone scan for staging.

Then the patients underwent a comprehensive breast ultrasound with colour and spectral Doppler in Esoate My Lab 60 ultrasound machine using high frequency probe (7 – 13 MHz).

In the ultrasound examination, the exact location of the lesion along with the measurements in the three dimensions are noted which enables us to calculate the volume of the tumor in cubic cm using the formula for calculation of volume of an ellipsoid as

Volume in cm^3 = d_1 x d_2 x d_3 x ∏/6

In the colour Doppler examination, the pulse repetition frequency was kept as low as possible to pick up even very slow flowing vessels. The wall filter was lowered to the maximum possible extent, such that it was just enough to avoid artefacts. The dynamic range was kept at 60. The optimal settings were selected for each patient individually at the baseline examination and the same settings were used in all further sequential examinations of a given tumour. Thus each patient acted as her own control. Then the number of colour signals noted within a standard colour box of area $1cm^2$completely filled with tumour was counted visually and recorded.

Then the spectral Doppler was switched on and the spectra were obtained in a portion of the vessel that is relatively straight. Care was taken to ensure that the waveforms obtained were continuous. The peak systolic velocity PSV, end diastolic velocity EDV and the resistivity index RI were measured. A minimum of three values were obtained. The lowest RI was taken for analysis.

Then the axilla was examined for lymph nodes and any abnormal node looking node (ie., those with round shape, loss of fatty hilum, diffuse or eccentric cortical thickening or ill defined margins) was noted.

CHEMOTHERAPY

All patients received 4 cycles of preoperative FAC chemotherapy once in 21 days as follows:

1. Inj. Cyclophosphamide 500 mg/m^2 intravenously on day 1
2. Inj. Doxorubicin 50 mg/m^2 intravenously on day1
3. Inj. 5-Fluorouracil 500mg/m^2 intravenously on day 1

Clinical examination, ultrasonography and Doppler examination were done at diagnosis before starting chemotherapy and repeated before every cycle of chemotherapy. At the end of four cycles of chemotherapy, a final clinical, sonographic and vascular response was recorded for every patient.

A scoring system based on the modified Response Evaluation Criteria In Solid Tumours (RECIST) v1.1 was employed for assessment of morphological response by clinical and sonographic evaluation depending on the change in tumour size from baseline as shown in table 1.[83] Both Progressive disease and stable disease according to RECIST were combined into the category of no response.

For Doppler, a scoring system modified from the one put forth by Kumar et al was used taking the RI and number of flow signals into

account. PSV was not included in our scoring system which is shown in table 2.

Table 1: Scoring system for clinical and sonographically assessed response (based on RECIST criteria version 1.1)

Score	**Response**	**% change from baseline (for clinical assessment- size of tumour, for sonography- volume of tumour)**
1	No response (NR)	Any increase or no change or a decrease of <30%in size/volume of tumour,
2	Partial response (PR)	Decrease of ≥30% in size/volume of tumour
3	Complete response(CR)	No clinically palpable tumour or sonographically detectable tumour at the end of chemotherapy

Table 2: Scoring system for Doppler assessment of response (modified from the scoring system proposed by Kumar *et al)*

SCORE	DEFINITION OF RESPONSE (RI)
1	Any decrease, no change or <25% increase in RI
2	25-50% increase in RI
3	50 - 75% increase in RI
4	>75% increase in RI or complete disappearance of flow signals
SCORE	**DEFINITION OF RESPONSE (number of signals/vessels)**
1	Any increase, no change or <25% decrease in feeder vessels
2	25-50% decrease in feeder vessels
3	>50% decrease in feeder vessels
4	Complete disappearance of flow signals

The scores obtained separately for RI and the number of flow signals at the end of chemotherapy were added to get a cumulative response assessment score by doppler as shown in table 3.

Table 3 : Cumulative Doppler Score

CUMULATIVE SCORE	RESPONSE
2-3	No change/increase in vascularity (no response)
4-6	Partial decrease in vascularity (partial response)
7-8	Complete disappearance of vascularity (complete response)

All patients underwent modified radical mastectomy (MRM) after completion of four cycles of chemotherapy and post operative tumour specimen was assessed for size, grade, lympho-vascular invasion and lymph nodal status. Final response to chemotherapy was documented as a percentage of change in tumour from baseline using the RECIST criteria.

The results obtained were tabulated in an Excel format. The response assessments on the basis of clinical examination, ultrasonography and Colour Doppler were compared with the histological response as gold standard and the assessment method correlating best with histopathology was analyzed using weighted kappa statistics, t tests and chi square tests with the help of the SPSS version 20 statistical software. A ‘p’ value of <0.05 was considered as significant.

RESULTS & STATISTICAL ANALYSIS

Out of 182 patients newly diagnosed with breast cancer during the study period, 63 patients had LABC, which is 34.6% of all breast cancers in our hospital during the period. Fifty four patients were eligible to enter the study as they satisfied the inclusion criteria. The other 9 patients were excluded as; 2 patients was not willing to undergo any treatment, one patient had rheumatic heart disease and was in failure with ejection fraction of <50%, 1 patient had grossly elevated renal parameters, two patients were aged above 75 years with performance status >2, and three patients were lost to follow up during chemotherapy.

The mean age of our patients was 50.9$\pm$ 8.3 years with a range of 27-65 years. 44% of patients were over 50 years (no. of patients=24) and one patient was below 30 years (2%). The results are shown in the table 4.

Table 4 : Age distribution of patients

Age (years)	No. of patients	Percentage
<30	1	2
30 – 50	29	54
>50	24	44
Total	54	100

47 patients (87%) were in tumour stage T4. Of these, Skin involvement alone (T4B) was seen in 45 patients (83%). Two patients had both skin and chest wall involvement (T4C) (4%). Seven patients (13%) had neither skin nor chest wall involvement and were in tumour stage T3. The tumour stage distribution at presentation is shown in table 5.

Majority (76%) of our patients presented with stage IIIB. Twenty percent of patients (11 patients) presented in stage IIIC. Two patients (4%) presented with stage IIIA. The tumour was in the right breast in 22 patients (40.3%) and in left breast in 32 patients (59.7%). The results are shown in table 6.

Table 5 : Tumour stage at presentation

Tumour stage	No. of patients	Percentage
T3	6	11
T4B	45	83
T4C	2	4
T4D	1	2
Total	54	100

Table 6 : TNM Stage at presentation

TNM stage	No. of patients	Percentage
IIIA	2	3.7
IIIB	41	75.9
IIIC	11	20.4
Total	54	100

In 28 patients (52%),at diagnosis, the nodal status was N1. 14 patients(26%) had N2 and 11 patients(20%) had N3. Only one patient had clinically node negative axilla, N0 disease (2%). The results are shown in table 7.

Table 7 : Nodal status at presentation.

Nodal status	No. of patients	Percentage
N0	1	2
N1	28	52
N2	14	26
N3	11	20
Total	54	100

The mean tumour size at diagnosis was 8.9 ± 3.2 cm. 34 patients (62.9%) had a tumour size of 5-10 cm. Thirteen patients had a tumour size of more than ten centimetres at diagnosis (24.1%) and 7 patients had a tumour size of less than 5 cm (13%). The tumour size at baseline is shown in table 8.

On B mode ultra sonogram, the mean volume of tumour was 106.3± 46.7 cc. At diagnosis, 8 patients had a tumour volume of over 150 cc (24.1%) whereas 13 patients had tumour volume of <75 cc (1.9%). Thirty three patients had a tumour volume of 75 - 150 cc (14.8%) at diagnosis. The tumour volume at diagnosis is shown in table 9.

Table 8: Tumour size at baseline (clinical examination)

TUMOR SIZE	**Number of patients**	**Percentage**
<5 cm	7	13
5-10 cm	34	62.9
>10 cm	13	24.1
TOTAL	54	100
Mean ± SD	**8.9 ± 3.2 (cms)**	

Table 9 : Tumour volume at baseline

Tumour volume by USG at baseline	Number of patients	Percentage
<75cc	13	24.1
75– 150 cc	33	61.1
>150 cc	8	14.8
TOTAL	54	100
Mean volume	**106.3 ± 46.7 cc**	

53 patients (98%) had infiltrating ductal carcinoma (not otherwise specified type). According to literature also, this is the most common histological subtype of breast cancer. One patient had infiltrating ductal carcinoma with neuroendocrine differentiation (2%).

Scarf-Bloom-Richardson score was used to calculate the grade of the tumour. Tumours of grade 2 were seen in 26 patients (48.1%), tumours of grade 1 in 16 patients (29.6%) and tumours of grade 3 in 12 patients (22.3%). This is shown in table 10.

Table 10: Tumour grade distribution

TUMOUR GRADE	Number of patients	Percentage
Grade 1	16	29.6
Grade 2	26	48.1
Grade 3	12	22.3
TOTAL	54	100

The estrogen and progesterone receptor status as well as Her-2/*neu*status were assessed from the histological sample. 37 patients (70%) were positive for ER/PgR and 15 patients (28%) showed Her-2 positivity which included both ER/PgR positive and ER/PgR negative tumours. All 3 markers were negative (Triple negative pattern) was seen in 12 patients (22.2%). The receptor status in the study group is shown in table 11.

Table 11 : Receptor status in the study group

RECEPTOR STATUS	N	%
ER and/orPgR +, Her-2/*neu*-	27	50.7
ER and/orPgR+, Her-2/*neu* +	10	18.2
ER-, PgR-, Her-2/*neu* +	5	8.9
ER-, PgR-, Her-2/*neu* –	12	22.2
Total	54	100

DOPPLER FEATURES:

A baseline doppler examination of the tumour was done at diagnosis and was repeated after each cycle. Majority of the tumours were hypervascular(>5 vessels within 1 cm^2 of the tumour). There was no predominant vascular pattern as most tumours had both central and peripheral vascularity.

The most striking feature of the intratumoral vessels was their tortuosity. Their course was marked with multiple sharp turns. This was important because it posed significant difficulty in correction of doppler angle when a doppler spectrum was to be obtained. Though care was taken to obtain all values from relatively straight portions of the vessels and angle correction was done to the best possible extent, the accuracy of doppler angle dependent velocity indices could not be assured owing to the extreme tortuosity. The values of the velocity indices showed a wide variation evidenced by the higher value of standard deviation relative to the mean.

Table 12 : Doppler findings

	Mean value at baseline	Mean value after cycle 4
PSV(cm/sec)	32.6±16.9	18 ± 16.3
EDV	14.2 ± 16.2	4.7 ± 6.6
RI	0.61 ± 0.14	0.64± 0.29
NUMBER OF SIGNALS	7.2± 2.2	1.6±1.5

The Doppler values (PSV, EDV, RI and number of flow signals) were tabulated at baseline and after every cycle of chemotherapy. The mean RI at baseline was 0.61 ± 0.14 which increased to 0.64± 0.29 after four cycles. The number of flow signals decreased from 7.2 ± 2.2 at baseline to 1.6 ± 1.5 at the end of chemotherapy.

The mean PSV measured at baseline was 32.6 ± 16.9 cm/sec, and at the end of four cycles it was 18 ± 16.3 cm/sec. The mean EDV at baseline was 14.2 ± 16.2 cm/sec which showed decrease to 4.7 ± 6.6cm/secafter 4 cycles of chemotherapy.

Table 13 : Change in doppler indices after chemotherapy

	PSV		EDV		RI		Vessels	
	N	%	N	%	N	%	N	%
No change	0	0	1	1.8	2	3.7	2	3.7
Increase	12	22.2	11	20.3	33	61.1	3	5.6
Decrease	42	77.8	42	77.8	19	35.2	49	90.7
Total	54	100	54	100	54	100	54	100

The changes in value of Doppler indices obtained at the end of chemotherapy were tabulated. An increase in RI was observed in 33 patients (61.1%) whereas 19 patients (35.2%) showed a decrease with chemotherapy. Two patients had no change in RI at the end of four cycles of chemotherapy. Compared to the baseline, the number of flow signals decreased in 49 patients (90%), increased in three patients (5.6%) and remained the same in 2 patients (3.7%). Forty two patients (77.8%) had a decrease in PSV and 12 patients (22.2%) had an increase. In 11 patients (20.3%) there was an increase in EDV and in 42 patients (77.8%) there was a decrease. One patient had no change in EDV at the end of therapy. The results are shown in table 13.

At the end of chemotherapy, 19 patients (35.2%) showed an RI score of 1 (<25% increase from baseline), 14 patients (25.9%) showed a score of 2 (25 to 50% increase from baseline), 12 patients(22.2%) showed a score of 3 (50 to 75% increase from baseline) and 9 patients (16.6%) showed a score of 4(>75% increase from baseline). 72% of patients showed >75% decrease in the number of flow signals at the end of chemotherapy (score = 3), whereas only 11% patients showed a complete disappearance of flow signals.

The cumulative Doppler score at the end of therapy was between 2-3 in 8 patients (14.8%), between 4-6 in 37 patients (68.5%) and 7-8 in 9 patients (16.7%).

HISTOPATHOLOGICAL FINDINGS

The post operative specimen was examined histopathologically and the results were tabulated. Eight patients (14.8%) showed pathological complete response in the primary tumour and 29.6% achieved nodal complete response. Nine patients showed no response to chemotherapy (16.7%). Combined response (both complete and partial response) rate was 83.3%.

Tumour size in the resected specimen was <2 cm in 20 patients (35%), 2 to 5 cm in 21 patients (42.1%), and >5 cm in five patients (9%). 39 patients (72.2%) showed lymphovascular invasion. Out of the 47 patients who had skin involvement at diagnosis, 44 patients (93.6%) showed resolution of skin invasion post chemotherapy. Chest wall involvement had resolved in both the patients who had chest wall involvement at diagnosis. The results are shown in table 14.

Post operative nodal status was N0 in 16 patients (29.6%), N1a in 26 patients (48.1%), N2a in 11patients (20.4%) and N3a in 1 patient (1.9%).

Table 14 :Histopathological findings

	N	**%**
Tumour size:		
0 cm	8	14.8
<2 cm	20	37
2-5 cm	21	38.9
>5 cm	5	9.3
Lymphovascular invasion	39	72.2
Skin involvement	3	5.5
Axillary lymph nodes		
0 nodes	16	29.6
<3 nodes	26	48.1
3-6 nodes	11	20.4
>7nodes	1	1.9

The pre-treatment clinical characteristics of the cases were analysed with respect to histopathological response. The results are shown in table 15.

Table 15: Pre-treatment clinical characteristics versus histological response

	HISTOPATHOLOGICAL RESPONSE		**P value**
	RESPONDERS (CR AND PR)	**NONRESPONDERS (NR)**	
Age:			
<50 years	20	5	0.807
>50 years	25	4	
Tumour size:			
<5 cm	3	4	
5-10 cm	32	2	**0.003**
>10 cm	10	3	
Tumour grade:			
Grade 1	13	3	
Grade 2	21	5	0.67
Grade 3	11	1	

In our study, the only clinical factor which correlated significantly with histological response was tumour size at baseline. Medium sized tumours of 5-10 cm had a significantly better response rate than tumours <5cm or >10 cm ($p<0.003$). Age of the patient, tumour grade and nodal status at presentation did not correlate with histological response.

The pre treatment volume of the tumour was compared with pathological response and the results were tabulated in the table 16. Tumours with a baseline volume between 75 and 150 cc had a better response rate than those with a volume less than 75cc or more than 150cc.

The baseline Doppler characteristics were compared with histopathological response as shown in table 17.

Table 16 :Pre treatment tumour volume versus histopathological response

Tumour volume by USG at baseline	**NON RESPONDERS**	**RESPONDERS**	**P value**
<75cc	5	8	**0.02**
75– 150 cc	2	31	
>150 cc	2	6	

Table 17: Pre-treatment Doppler characteristics versus histological response: mean values

	Responders	**Non responders**	**p value**
Mean baseline PSV	31.9± 16.5	35.6± 19.8	0.55
Mean baseline EDV	22.2 ± 18.7	31.9± 32.7	0.22
Mean baseline RI	0.59 ± 0.14	0.70± 0.09	**0.028**
Mean number of feeder vessels at baseline	7.7 ± 2	4.7± 1.5	**<0.0001**

It was observed that the mean number of feeder vessels was significantly lower in tumours which did not respond to chemotherapy (4.7± 1.5) compared to tumours which responded well to chemotherapy (7. 7 ± 2). The baseline mean RI was also found to be significant with tumours having a lower pre-treatment RI showing better response (p=0.039). The baseline mean PSV and EDV values were not significantly different between responders and non responders.

Table 18: Baseline RI and feeder vessels versus response: absolute values

	HISTOLOGICAL RESPONSE		P VALUE
	NON RESPONDERS	**RESPONDERS**	
RI			
<0.5	0	10	
0.51-0.70	5	27	0.109
>0.70	4	8	
Total	9	45	
Vessels	**Non Responders**	**Responders**	
<5	7	6	
6-8	1	13	**0.0002**
>8	1	26	
Total	9	45	

The number of signals observed at baseline was very significantly different, with tumours having fewer than five feeder vessels having a tendency for non responsiveness to chemotherapy (p=0.0002). The absolute baseline RI values also showed significant difference between responders and non responders (p=0.03). The baseline PSV and EDV did not correlate with response (p=0.231).

The grade of the tumour determined by the Scarf Bloom Richardson scoring system was compared with the baseline RI values. The baseline RI values had a good correlation with the tumour grade, with grade 1 tumours having a higher RI than the grade 3 tumours. An RI cut off of 0.70 had a good predictive value (83%) for grade 1 tumours. The correlation was statistically significant ($p<0.00001$). But both baseline RI and grade of the tumour did not show any significant correlation with histological response. The results are shown in table 19.

Table 19: Baseline RI score versus grade of tumour

	GRADE 1	**GRADE 2**	**GRADE 3**	**TOTAL**
<0.50	0	3	9	12
0.51-0.70	6	22	2	30
>0.70	10	1	1	12
TOTAL	16	26	12	54
P VALUE = <0.0001				

We found that the change in RI after 4 cycles of chemotherapy correlated significantly with histological response, with a higher number of

non responders having an RI score of 1 (<25% increase from baseline). The results were statistically significant (p<0.0001) and are tabulated in table 20.

Table 20: RI score versus histological response

RI score at end of chemotherapy	**Histopathological response**				**P value**
	NR	PR	CR	Total	
1	7	12	0	19	**<0.0001**
2	2	10	2	14	
3	0	12	0	12	
4	0	3	6	9	
Total	9	35	8	54	

COMPARISON OF RESPONSE

Clinical, sonographic, vascular and pathological response for patients were tabulated and compared. 8 patients achieved pathological complete response which is the gold standard. Complete response was seen in 21 patients as per clinical assessment, 8 patients in ultrasound assessment and 9 patients as per doppler assessment The results are shown in table 21.

Table 21: Comparison of clinical, sonographic, vascular and histological response

RESPONSE	Clinical response		Sonographic response		Vascular response		Pathological response	
	N	%	N	%	N	%	N	%
NR	9	16.6	12	22.2	8	14.8	9	16.7
PR	24	44.4	34	63	37	68.5	37	68.5
CR	21	36.8	8	14.8	9	16.7	8	14.8
TOTAL	54	100	54	100	54	100	54	100

Sixteen percent (n=9) of patients had no response according to clinical assessment whereas 23% (n=13) according to ultrasound and doppler assessment and 18% (n=10) according to histological assessment had no response.

Response assessed by clinical, sonographic and Doppler methods was compared compared with the gold standard of pathological response individually.

The clinical and histological responses are compared in table 22. Only seven patients out of 21 who had CR by clinical examination, had pathological CR. Of the remaining, 12 had only PR and 2 had NR on HPE. Of the 10 patients

who had NR on clinical assessment, 6 patients had NR and 4 patients had PR on HPE. Out of the 26 patients who had PR on clinical examination, 23 had PR on HPE also; 1 patient had CR and 2 patients had NR on HPE.

Table 22: Clinical response versus histological response

CLINICAL RESPONSE	HISTOLOGICAL RESPONSE			TOTAL
	NR	PR	CR	
NR	5	4	0	9
PR	2	21	1	24
CR	2	12	7	21
TOTAL	9	37	8	54

The ultrasound and histological responses are compared in table 23. Out of nine patients who had CR as per sonographic assessment, three patients (37.5%) had CR, four patients had PR and one patient had NR on HPE. Five patients (62.5%) who had pathological complete response were found to have partial response by sonographic assessment. This may be due to the presence of fibrotic mass after chemotherapy which is taken as residual tumour mass at ultrasound but contains no viable tumour cells on histological examination. Eight out of the thirteen patients who had no response on sonography had no response in HPE and the remaining 5 had partial response. Out of the 36

patients who had PR on sonographic assessment, 30 patients had PR on HPE also.

Table 23: Sonographic response versus histological response

SONOGRAPHIC RESPONSE	HISTOLOGICAL RESPONSE			TOTAL
	NR	PR	CR	
NR	7	5	0	12
PR	1	28	5	34
CR	1	4	3	8
TOTAL	9	37	8	54

The doppler and histological responses are compared in table 24. We found that, of the 9 patients who had CR on Doppler assessment, six patients had CR and three patients had PR on histological assessment. Eight (88.9%). Out of the 9 patients who had no response in Doppler assessment, had no response on HPE also.

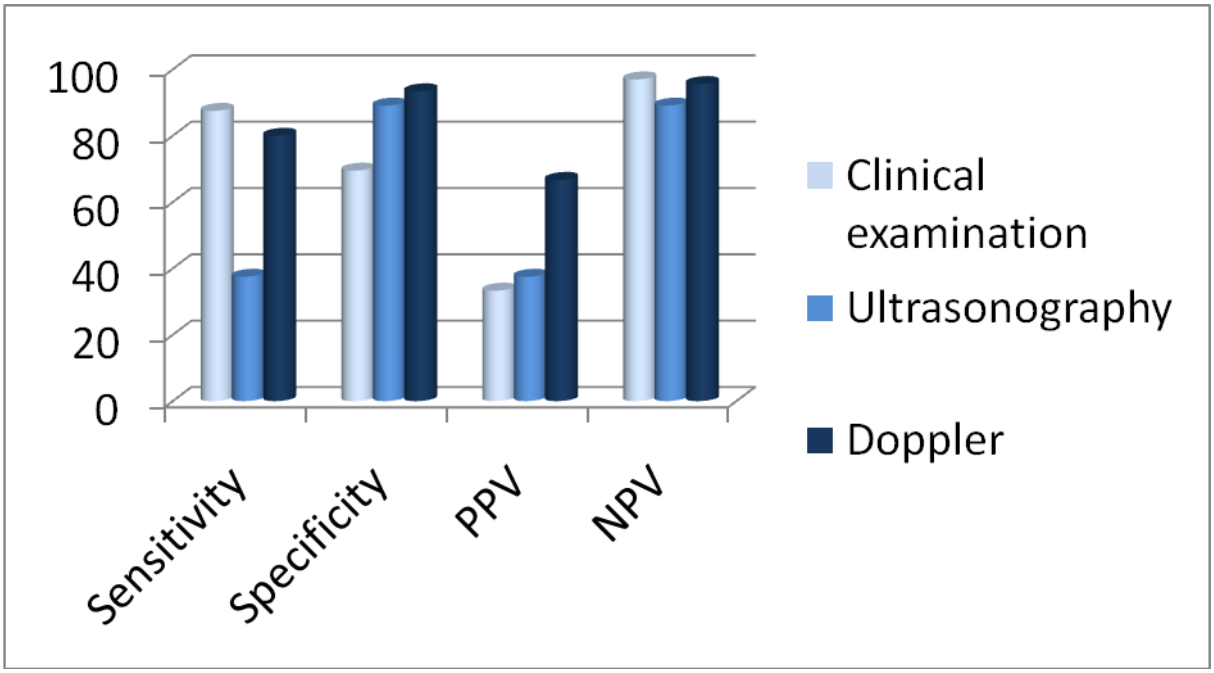
Table 24: Vascular response versus histological response

VASCULAR RESPONSE	HISTOLOGICAL RESPONSE			
	NR	PR	CR	TOTAL
NR	7	1	0	8
PR	2	33	2	37
CR	0	3	6	9
TOTAL	9	37	8	57

The sensitivity, specificity and predictive values of various modalities for predicting a response on histology at the end of therapy was calculated. We found that although clinical examination had the highest sensitivity (87.5%) for predicting CR, it had a lower specificity (71.4%) than B mode ultrasound (89.8%) or Doppler (93.8%). Both clinical examination and Doppler had a very high negative predictive value for prediction of CR (97.2% and 95.8% respectively). It was also observed that the positive predictive values of clinical examination and sonography were low (33.3% and 37.5% respectively) compared to Doppler (66.6%). The results are shown in table 25.

Table 25: Sensitivity and Specificity of the modalities for predicting a pathological CR.

	Sensitivity	Specificity	PPV	NPV
Clinical examination	87.5%	69.5%	33.3%	97%
Sonography	37.5%	89.1%	37.5%	89.1%
Doppler	80%	93.4%	66.7%	95.6%



The sensitivity, specificity and predictive values for the various modalities for prediction of lack of response on histopathology were calculated and the results were tabulated in table 26. Doppler had a very high specificity and positive predictive value (97.8% & 88.9%) compared to clinical examination

(91.4% & 60%) and B mode ultrasound (89.3% & 61.5%). The sensitivity and NPV of Doppler was almost equal to that of B mode ultrasound.

Table 26 : Sensitivity and specificity of the modalities for predicting non responders on histopathology

	Sensitivity	Specificity	PPV	NPV
Clinical examination	55.6%	91.1%	55.6%	91.1%
Sonography	77.8%	88.9%	58.3%	95.2%
Doppler	77.8%	97.8%	87.5%	95.6%

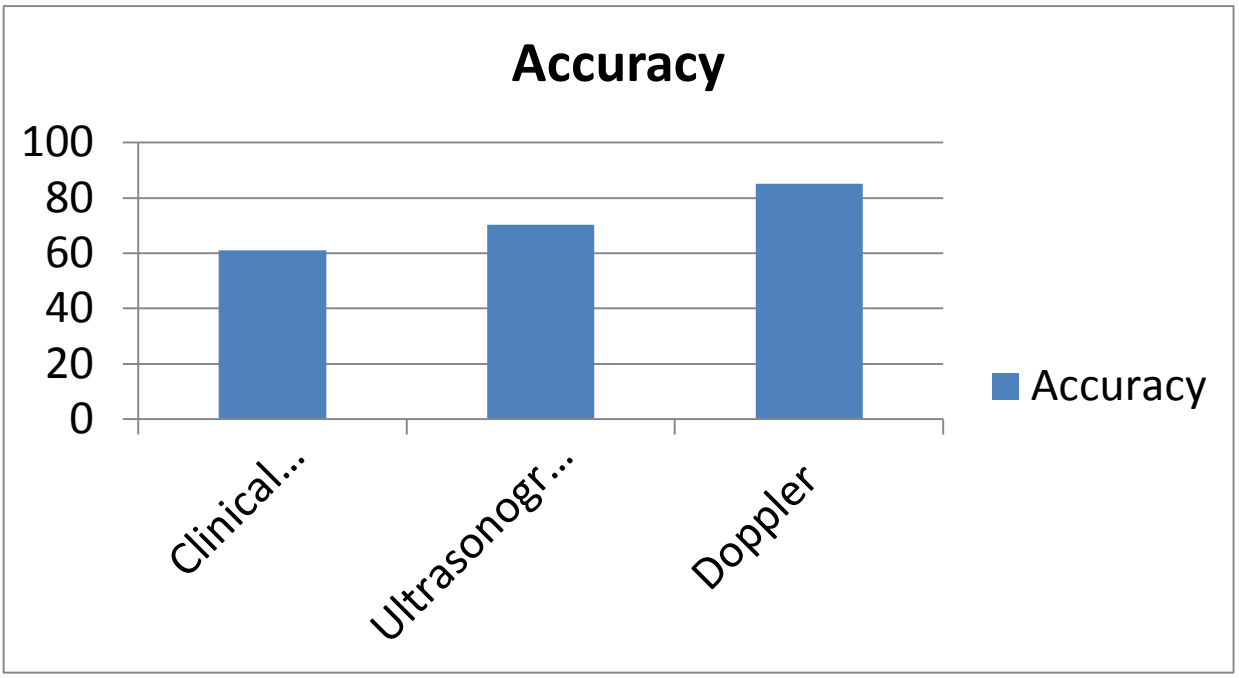
A cumulative Doppler score of >3 after completion of chemotherapeutic regimen had a high sensitivity (90%), specificity (90%) and positive predictive value (98%) for prediction of response (both CR and PR). Similarly, a cumulative Doppler score of >6 was highly predictive of pathological complete response.

The accuracy of prediction of response was compared for all three modalities in table 27. The accuracy of response prediction was 61.1% for clinical examination, 70.3% for ultrasound and 85.1% for Doppler.

Table 27 : Accuracy of response prediction.

	No. of correctly predicted non responders	No. of correctly predicted partial responders	No. of correctly predicted complete responders
Clinical examination	5	21	7
Ultrasound	7	28	3
Doppler	7	33	6

	Total number of accurate results	Total number of patients	Accuracy
Clinical examination	33	54	61.1%
Ultrasound	38	54	70.3%
Doppler	46	54	85.1%



AGREEMENT – WEIGHTED KAPPA STATISTICS

Using weighted kappa statistics, agreement between the response obtained by clinical, sonographic and vascular assessment and pathological assessment was calculated and results were tabulated. It was found that the agreement between vascular and histological response was the best of the lot (κ =0.71). Agreement between clinical and histological response was the least (κ =0.39). The agreement between ultrasound response and pathological response was intermediate (κ=0.48). The agreement was rated as fair, moderate or good, according to the definition of the kappa agreement statistics.

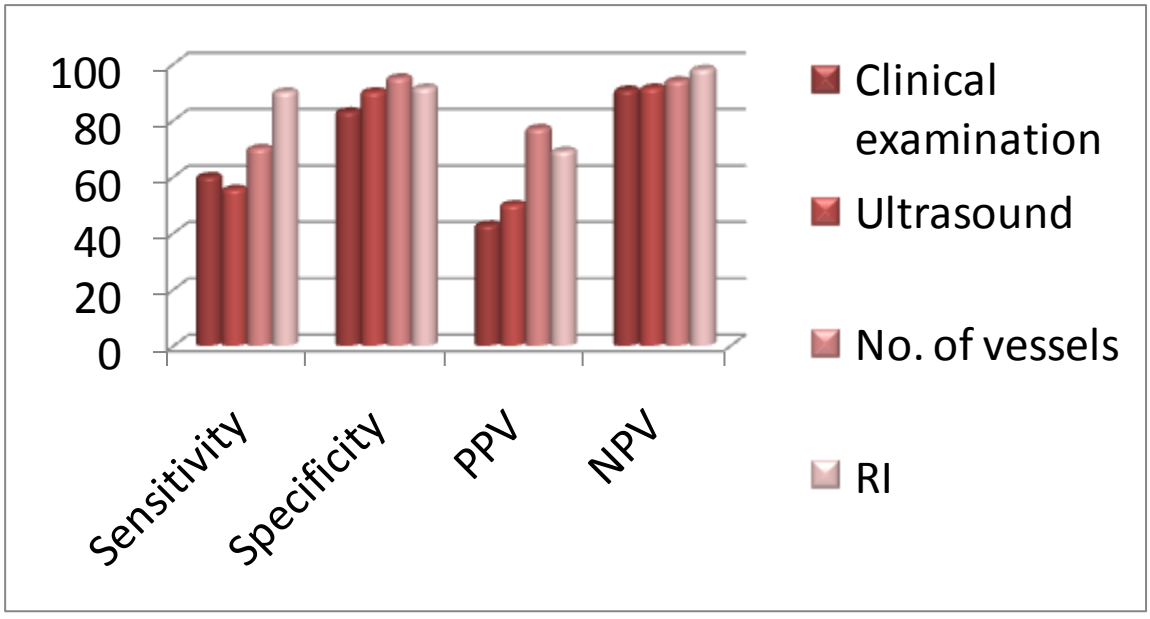
Table 28: Agreement between clinical, sonographic, vascular response and pathological response (weighted kappa statistics)

	K (95% CI)	**P value**	**Agreement**
HPE and clinical response	0.39 (0.19-0.58)	<0.0001	FAIR
HPE and sonographic response	0.48 (0.23-0.73)	<0.0001	MODERATE
HPE and vascular response	0.77 (0.60-0.92)	<0.0001	GOOD

Prediction of non responders to chemotherapy was done at interim assessment (after the completion of two cycles of chemotherapy), using a criterion of failure to achieve at least a 25% expected change from baseline. The results are summarized in table 29.

Table 29: Prediction of non responders after 2nd cycle chemotherapy using 25% change from baseline as cut off

	SENSITIVITY	SPECIFICITY	PPV	NPV
Clinical	55.6%	82.2%	38.5%	90.2%
USG	66.7%	86.7%	50%	92.8%
RI	90%	84.4%	53.3%	97.4%
Feeder vessels	66.7%	93.3%	66.7%	93.3%



The change in RI had high sensitivity and specificity for predicting non responders (90% and 91.4% respectively) with a high negative predictive value (98%). Failure of the vascular signals to decrease by 25% after two cycles also had a high specificity and sensitivity (70% and 95% respectively) for predicting non responders with a negative predictive value of 94%. Clinical examination and sonography also had high negative predictive values but their sensitivity and specificity values were low when compared to those of Doppler. The accuracy of prediction was good for Doppler with 79%.

DISCUSSION

The incidence of LABC in our hospital which is a tertiary care centre is 34.6%. This is concordant with the incidence levels in developing countries (30 – 60%).[83] There was no significant difference in the response rates of women below 45 years of age as against those above 45 years. The tumour size and receptor status showed a varied pattern without any predominant pattern.

The ER/PgR positivity rate was 68% which was comparable to those in western countries.[83] The triple receptor negative subset constituted 22.2% which concurred with the world figures (15 – 25%).

The overall response rate was 83.3%.This is in concordant with many studies whish have reported response rates of 85 – 90% to primary systemic chemotherapy especially with anthracycline based regimen like our study. The rate of pathological complete response is 14.8%.

Nine patients (16%) did not respond to chemotherapy while the reported rate of non responders is 3 – 20% in various studies. Though these 9 patients did not respond to chemotherapy in terms of tumour size reduction or vascularity, all of them achieved resolution of skin and chest wall invasion, making them operable. This adds to the evidence regarding the usefulness of neoadjuvant chemotherapy in inoperable breast malignancy.

Tumour size had a significant relationship with response to chemotherapy with tumours smaller than 5 cm and larger than 10 cm showing a less favourable response when compared to tumours between 5 and 10 cm in size. Also the tumours which had a volume between 75 and 150 cc had a better response rate than those with volumes below 75cc or above 150cc. This is in contrast to Gajdos et al who reported better response rates in smaller tumours.[85]

Clinical assessment overestimated complete response in 26% . . Sun et al reported a false complete remission rate of 46.8% while in our study it was 66.7%. While considering the ability of clinical examination to predict overall response, it had a low positive predictive value (33.5%) and specificity (69.5%) though the negative predictive value (97%) and sensitivity (87.5%) were very high. Also it underestimated response in 44% of cases.[22] This trend is seen in other studies also. Feldman et al and Cocconi et al have also reported higher false positive rates in assessment of response by clinical methods.[14,21] Khoker et al found that the initial clinical response after the first cycle of chemotherapy predicted final clinical response well. But out of the 15 patients who showed initial clinical response, only 6 (40%) patients achieved pathological complete response.[18] von Minckwitz et al have reported that early clinical response (after the second cycle) is good predictor for pathological complete response but it was not an independent predictor.[20]

The problem with clinical examination is that small areas of residual viable tumour cells may not be palpable. The presence of fibrotic reaction makes it even more difficult to identify small residual masses by palpation. Also, it is impossible to differentiate a fibrotic mass and post intervention inflammation from tumour mass.

B mode ultrasonography is not a reliable method of assessment of both pathological complete response and lack of response. It correctly identified only 3 out of the eight complete responders (37.5%). As B mode ultrasound measures only the size of the tumour, in cases where the tumour size remains the same in spite of it being replaced by non viable fibrotic or necrotic tissue due to the effect of chemotherapy, ultrasound is not able to identify the response. Its ability to identify non responders is limited by the changes in echogenicity of the tumour after chemotherapy, which may make it difficult for the examiner to differentiate the tumour from the surrounding tissue. The tumour may become isoechoic to the surrounding tissue or may develop afibrotic reaction in the surrounding tissue making the distinction of tumour margins unidentifiable in either case.

Imaging the vascularity of tumours helps us avoid the pitfalls of morphological imaging. As tumour angiogenesis is a very important phenomenon in tumour survival and growth, changes in vascularity closely reflect the change in the viability of tumour cells. Thus were are able to identify

correctly, the absence of tumour tissue in a clinically palpable, sonographically visible mass and the presence of viable tumour in an area which is impalpable and sonographically unidentifiable.

Doppler, which is the least expensive method of imaging vascularity, is the only practical option in less well developed countries which are short of the monetary resources required to battle the rising incidence of cancer. The ability to predict the lack of response early not only helps us save the patient from the toxic effects of the ineffective drugs and offer her alternatives for better disease control but also prevent wastage of expensive chemotherapeutic drugs to which some other patient would be sensitive and therefore be benefitted.

It has been proven that the interstitial pressure of the tumour microenvironment is increased due to accumulation of osmotically active macromolecules in the interstitium caused by the increased vascular permeability of tumour vessels.[86] As the tissue oncotic pressure (interstitial pressure) reaches the level of hydrostatic pressure of blood vessels, the tissue perfusion pressure becomes almost nulled as according to Starlings hypothesis,

Tissue perfusion pressure = hydrostatic pressure – tissue oncotic pressure.

Further increases in interstitial pressure cause the tumour blood vessels to collapse by mechanical compression.

It could be construed that this increase in interstitial pressure causing resistance to flow is reflected by resistivity index in Doppler. But this effect could be counter balanced by the arteriovenous shunts which known to be are abundant in the tumour vessels. When AV shunts are present the blood is not exposed to the the resistance of the increased IFP and instead channelized into the low resistance pathway, the veins.

But the tumour microenvironment is very heterogeneous. So a tiny area of increased interstitial pressure could be adjacent to an area of normal interstitial pressure. This could be the reason why there are conflicting reports about the RI values in malignancies.[77,79,87]

In our study, the baseline RI was significantly different between the responders and non responders with the non responders having a higher RI. This may be explained by the fact that drug delivery to tumours having an increased interstitial fluid pressure is less than optimal. Vavra et al have proved that intravenously injected ^{14}C-labelled sucrose was concentrated in the necrotic areas and surrounding normal tissue (where the interstitial pressure is lower) rather than the areas of viable tumour.[87]

In our study, we took multiple measurements and used the lowest RI for analytical purpose following Osanai et al. The lowest RI could represent an area where the interstitial pressure was not high enough to cause substantial increase in vascular resistance. The baseline RI had a significant correlation with the

histological grade of the tumour with higher grade tumors having a lower RI. This is in concordance with a study by Osanai et al where they found that grade 3 tumours had a lesser RI than grade 1 tumours.[73]

The complete disappearance of vascular signals was an independent predictor of pathological complete response with a specificity of 98% and positive predictive value of 83.3%. Kumar et al have recently reported the value of this finding. While counting the number of colour signals within an area of 1 cm^2 is cumbersome when there is no dedicated software, gross reductions in flow can be made out reliably on visual inspection by an experienced sonologist.[71] Huber et al have stated that a change in vascularity of the magnitude of 5% can be detected visually.[29]

In our study there was a uniform increase in RI in patients who responded to chemotherapy. In many other studies there was no dominant increase or decrease in RI with therapy that was significantly different between responders and non responders.[71,72] Oksuzoglu et al have reported that there was a uniform decrease in RI in responders in the 21 patients of LABC studied.[77] But many studies conducted in patients with locally advanced carcinoma of the cervix have reported that there was an an increase in RI with response to chemoradiotherapy.[80,81]

It could be postulated that in tumors that respond to chemotherapy, the interstitial fluid may not be very high (as very high interstitial fluid pressure is

an important impediment to optimal drug delivery).[88] (In our study too, we found that non responders had a higher baseline RI). In these cases the AV shunting in the tumour vasculature may play a substantial role in the flow resistance. With normalization of the vasculature with response to chemotherapy, the AV shunting is reduced and there is an increase in RI. This hypothesis needs to be verified by experiments on tumour microenvironment.

The vascular response based on the cumulative doppler score at the end of chemotherapy had a good sensitivity, specificity and negative predictive value for prediction of both complete responders (80%, 93.5% and 96%) and non responders (78%, 98% and 96%). It scored far better than clinical examination and B mode ultrasonography. Similar results have been obtained by many of the recently published studies. Kumar et al reported a sensitivity of 66% for doppler on comparison with 45% for clinical examination.[71] Kedar et al and singh et al have also reported good sensitivity of doppler to predict pathological response.[65,72]

The agreement rate between pathological response and doppler assessment has been found to be very good with a kappa value of 0.77. This surpassed the agreement between HPE and clinical examination and B mode ultrasonography. This is in concordance with Huber et al who found substantial agreement between doppler and HPE with a kappa value of 0.87.[29] Balu

maestro et al have also reported a high correlation between doppler and pathological response.[30]

The accuracy of doppler assessment which reflects the proportion of correct predictions is the highest for doppler at 85%, when compares to 61% for clinical examination and 70% for B mode ultrasonography.

The time and effort involved in any method employed to predict chemotherapeutic response is justified only when it is cost effective and clinically applicable and beneficial in the management of patients..

The main aim of this study was to be able to predict the response or the lack thereof to chemotherapy by an interim assessment before the completion of the regimen. Only then would we be able to make meaningful changes in the treatment of individual patients thus maximizing the benefit and minimizing the cost of therapy. In our study we assessed the response after each cycle in order to determine whether such early prediction using an inexpensive method like Doppler was possible and if possible to determine how early the response could be reliably predicted. We found that Doppler was very advantageous in this respect. We assessed clinical examination and B mode ultrasound alongside the Doppler parameters of RI and number of flow signals per unit area at the end of second cycle of chemotherapy. The cut off taken was the failure to achieve a 25% change from the baseline value. In case of RI, the expected change was an

increase with tumour response while all other parameters were expected to decrease following tumour response to chemotherapy.

We found that the Doppler parameters scored better than clinical examination and B mode ultrasound in sensitivity, specificity, positive predictive value and negative predictive value. RI had a better sensitivity and negative predictive value (90% and 97% respectively) while the number of flow signals per unit area had a better specificity and positive predictive value (93% and 67%).

RI can be measured easily in the tumour vessels if they can be visualised. The tortuosity of the tumour vessels donot hamper the measurement of RI as it would the velocity measurements, which are angle dependent.

Peak systolic velocity measurements were not useful in predicting the response in our study while Singh et al have reported decrease in PSV with response to chemotherapy. The main pitfall encountered by us in PSV measurement was due to the extreme tortuosity of the tumour vessels making angle correction almost impossible.

There were a few limitations to this study.

We did not use any specialized software for the quantification of number of flow signals per unit area of the tumour. Also power doppler was not used. These facilities involved additional expenditure and our study was aimed

primarily at cost reduction. In spite of these disadvantages this study shows that a basic colour doppler gives valuable information and reliably predicts the response early during the course of chemotherapy.

In this study comparison of doppler with dynamic contrast enhanced MRI which is now considered as the imaging gold standard, could not be done as it involved huge expense.

The accuracy of the velocity measurements could not be assured as the tumour vessels seldom followed a course straight enough to correct the doppler angle. In measuring the RI, the lowest value out of the multiple measurements taken was used for analysis, the rationale behind it being the vessel with the lowest RI was the main supplier as it showed the lowest resistance to blood flow. Osanai et al have used a similar concept in their study.[73]

In our study we considered the response of only the primary tumour and did not consider doppler imaging of the axilla. The nodal response is also considered a very important prognosticator and so further studies should be done to evaluate the doppler assessment of nodal response too.

Ultrasonography and doppler are very operator dependent. In order to reduce the inter observer variability, all the doppler examinations in this study was done by a single examiner. The clinical assessment was also done by a single person for all cases.

Doppler ultrasonography has been found to be a non invasive, relatively inexpensive and reproducible method of assessment of response to primary systemic chemotherapy in patients with locally advanced breast cancer. Since it directly visualizes the vessels and assesses tumour vasculature both qualitatively and quantitatively, it is a good method to image neoangiogenessis and vasculogenesis. It not only correlates very well with histopathological response, but also predicts the pathological response with good accuracy as early as after 2 cycles of chemotherapy. Though in this study assessment was done after every cycle, based on the results obtained, we suggest that a pre treatment baseline assessment and an interim assessment at the end of second cycle would suffice for accurate response prediction.

SUMMARY

The study titled “Doppler ultrasound assessment of tumour vascularity in locally advanced breast cancer at diagnosis and following primary systemic chemotherapy” was a prospective study of 54 patients with newly diagnosed LABC admitted in Rajiv Gandhi Government General Hospital, Chennai. Eligible patients underwent clinical examination, ultrasonography and colour doppler imaging with measurement of doppler indices at baseline and prior to every cycle of chemotherapy. All patients underwent definitive mastectomy after four cycles of chemotherapy with documentation of tumour size on pathological specimen. The correlation between various modalities of response

assessment and histopathological results was analysed. There were no significant pre-treatment clinical characteristics that predicted response. High vascularity at baseline correlated with good response. The cumulative Doppler score employed in this study was useful in identifying responders and non responders as early as post second cycle of chemotherapy with a high sensitivity and specificity. Doppler imaging had a better correlation with histology compared to other modalities.

This study highlights the fact that combining Doppler imaging with the routine clinical examination for response assessment could increase the sensitivity and specificity of both the methods and can identify non responders earlier.

CONCLUSIONS

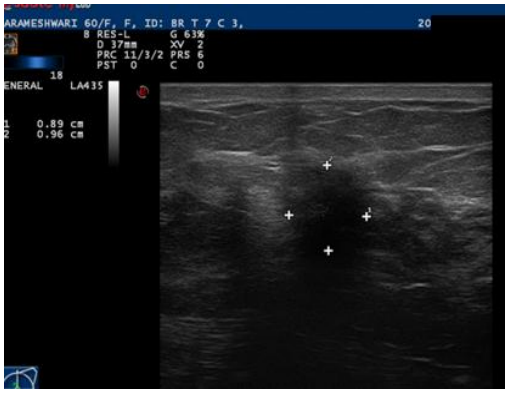
1. Colour Doppler imaging was a better modality for response assessment than clinical examination or ultrasonography.
2. Assessment by Doppler imaging had highest correlation with histopathology.
3. A cumulative score based on Doppler indices had a high specificity and sensitivity for identifying non responders and responders.
4. Hypervascular tumours had better response to chemotherapy than hypovascular tumours.
5. Failure of vascularity to decrease at least by 25% after the second cycle of chemotherapy had a high sensitivity, specificity and positive predictive value for non responsiveness to chemotherapy.
6. Failure of resistive index to increase during chemotherapy had a high sensitivity, specificity and positive predictive value for identifying non responders.
7. A baseline, interim and post chemotherapy Doppler imaging is useful in identifying non responders accurately.
8. Combining Doppler imaging with clinical examination could accurately predict response in most cases, hence can be recommended in the routine assessment of response.

Morphological responder showing decrease in tumour size on chemotherapy

Before chemotherapy

Size – 3.11 x 1.97 x 2.66cm

Vol - 16.3 cc



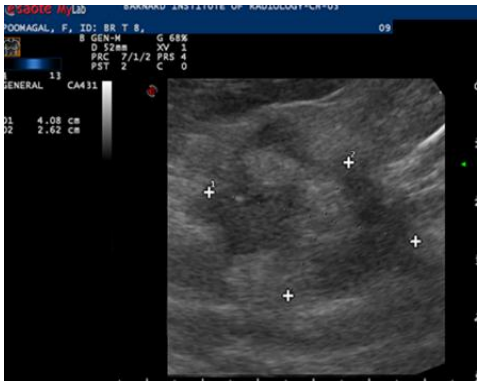
After cycle III

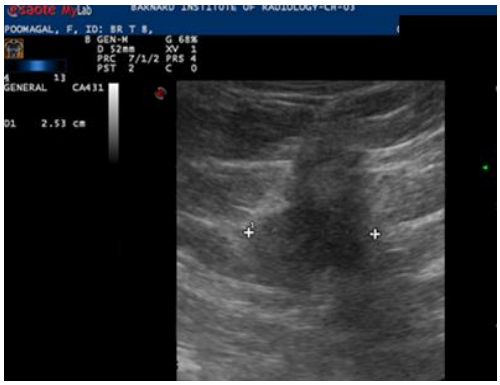
Size -1.48 x 0.89 x 0.96 cm

Vol - 1.26cc

92% decrease

Morphological non-responder showing increase in tumour size following chemotherapy.

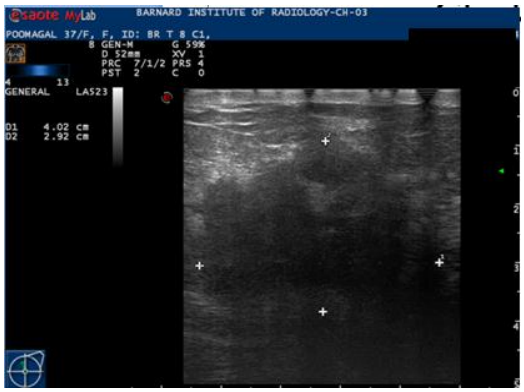




Before chemotherapy

Size – 4.08 x 2.62 x 2.53cm

Vol - 27.0cc



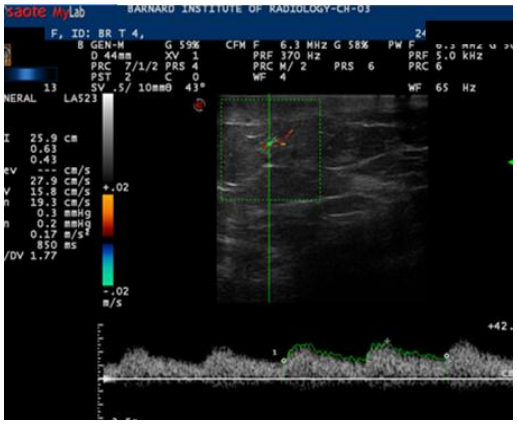
After cycle III

Size – 4.02 x 2.92 x 3.27 cm

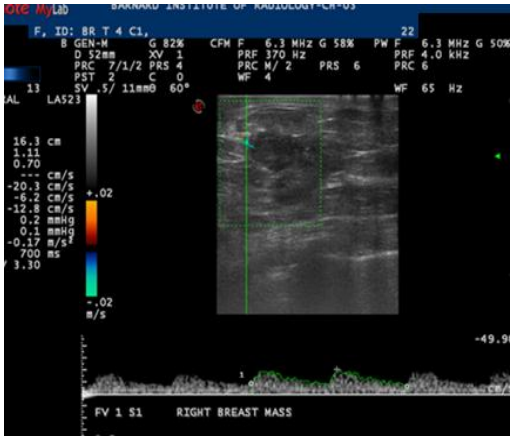
Vol - 38.4cc

42% increase

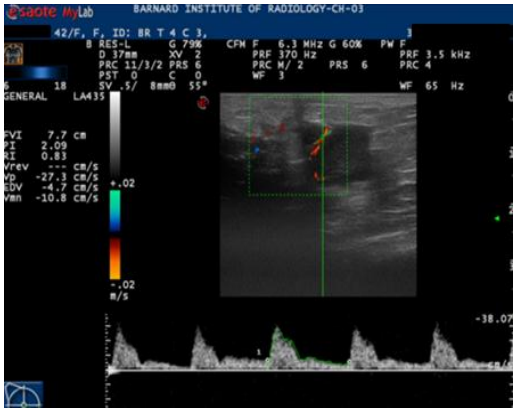
Vascular responder showing serial increase in RI following chemotherapy



Before chemotherapy
RI – 0.43

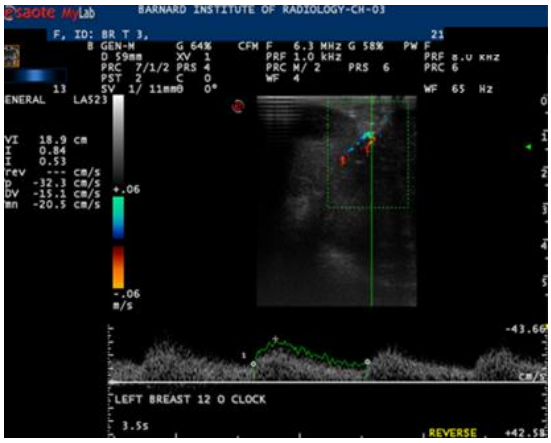


After cycle I
RI – 0.70
65% increase

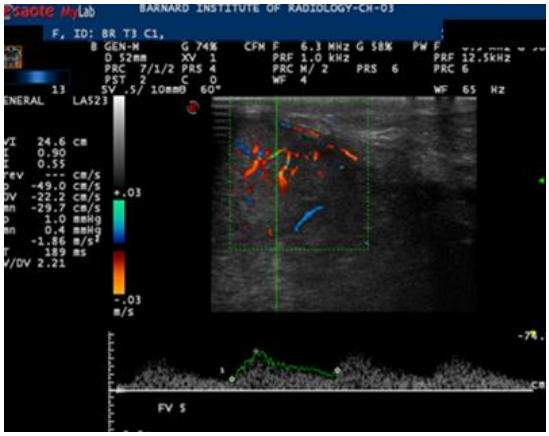


After cycle II
RI – 0.83
93% increase

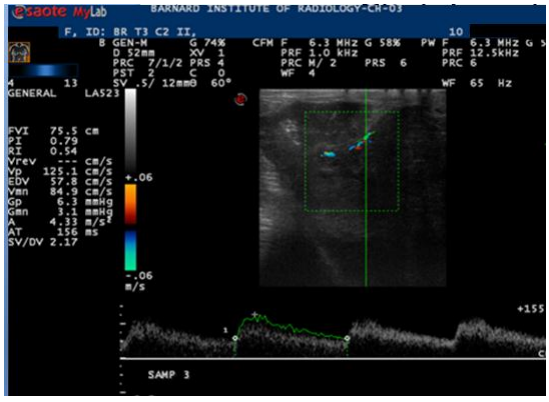
Vascular non-responder showing no significant change in RI



Before chemotherapy
RI – 0.53

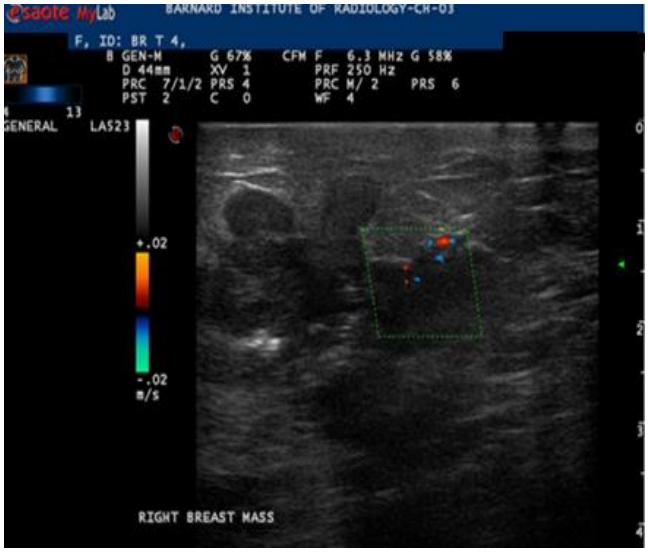


After cycle I
RI – 0.55



After cycle II
RI – 0.54

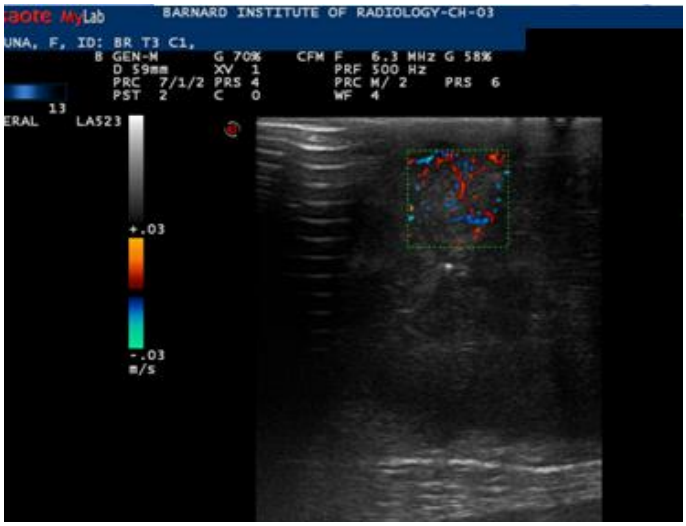
Vascular responder showing decrease in flow signals following chemotherapy



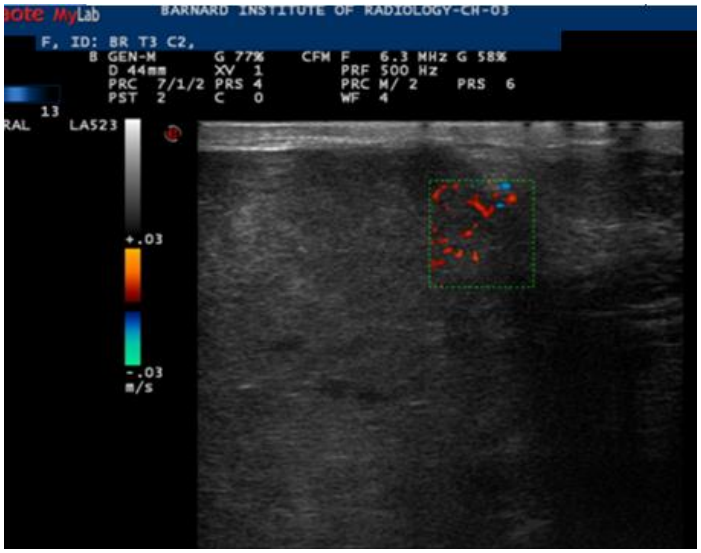
Before chemotherapy
7 signals

After cycle II
2 signals
71% decrease

Vascular non-responder showing no significant decrease in flow signals



Before chemotherapy
22 signals



After cycle II
21 signals
5% decrease

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INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr.R.S. Aarathhi Dhevi
PG in MD Radiodiagnosis
Madras Medical College
Chennai -3

Dear Dr.R.S. Aarathhi Dhevi

The Institutional Ethics committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled "Doppler ultrasound assessment of tumour vascularity in locally advanced breast cancer at diagnosis and following primary systemic chemotherapy " No.27072012.

The following members of Ethics Committee were present in the meeting held on 24.07.2012 conducted at Madras Medical College, Chennai -3.

1. Dr. S.K. Rajan. M.D.,FRCP.,DSc -- Chairperson
2. Prof. Pregna B. Dolia MD -- Member Secretary
   Vice Prinicpal, Madras Medical College, Chennai-3
   Director , Inst. of Biochemistry, MMC, Ch-3
3. Prof. Kalaiselvi MD -- Member
   Prof of Pharmacology ,MMC, Ch-3
4. Prof. C. Rajendiran, MD -- Member
   Director , Inst. of Internal Medicine, MMC, Ch-3
5. Prof. MD Ali M.D,. D.M., -- Member
   Prof & HOD, Dept. of MGE, MMC, Ch-3
6. Prof. S. Deivanayagam MS -- Member
   Prof of Surgery, MMC, Ch-3
7. Thiru. S. Govindsamy. BABL -- Lawyer
8. Tmt. Arnold Soulina MA MSW -- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/ Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

INFORMED CONSENT FORM

Title of the study: "Doppler Ultrasound Assessment Of Tumour Vascularity In Locally Advanced Breast Cancer At Diagnosis And Following Primary Systemic Chemotherapy".

Name of the principal investigator DR. R.S.Aarathhi Dhevi

Post graduate, M.D(Radiodiagnosis)

Madras Medical College.Chennai-3

Name of the institution: Rajiv Gandhi Government Hospital, Chennai-3

1. I understand that if I am found eligible, I may undergo certain extra tests and special studies which in any way do not affect my final report or management.
2. I have read and understood this consent form and the information provided to me.
3. I have been explained the nature of the study
4. I have had the consent from explained to me.
5. My rights and responsibilities are explained to me by the investigator.
6. I agree to cooperate with the investigator and inform her if I have unusual symptoms
7. I am aware of the fact that I can opt out of the study at any time without any reason and I am assured that this will not affect my treatment in this hospital
8. I hereby give permission to the investigator to release the information of the study and I will not have any control over the results of this study
9. I am giving my consent in full consciousness for this study to undergo clinical examination, breast ultrasonogram and color doppler study.
10. I give my consent to undergo chemotherapy and surgery when required, the nature of illness and consequences of treatment have been clearly explained to me.

11. I give permission to use my results wherever required.

12. My identity will be kept confidential if my data are publicly presented

I was free to ask questions and they have been answered. I am over 18 yrs of age and exercising my free power of choice, here by I am giving consent to be included as participant in the study titled “Doppler Ultrasound Assessment Of Tumour Vascularity In Locally Advanced Breast Cancer At Diagnosis And Following Primary Systemic Chemotherapy”.

Patient’s signature/thumb impression: date:

Name and address:

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு:

மிகவும் தீவிரமான மார்பகப் புற்றுநோய் உள்ள நோயாளிகளில் புற்றுநோய் மருந்து சிகிச்சைக்கு முன்னும் பின்னும் "கலர் டாப்லர்" மூலம் சிகிச்சையின் பயன்பாடு பற்றி அறிதல்.

பெயர்: தேதி:

வயது: உள்நோயாளி எண்:

பால்: ஆராய்ச்சி சேர்க்கை எண்:

1. நான் இந்த ஆய்வுக்கு தகுதியானவராகக் கருதப்பட்டால், சில சிறப்பு பரிசோதனைகளுக்கு உட்பட நேரிடும் என்றும், அத்தகைய பரிசோதனைகள் எனது இறுதி அறிக்கையையோ அல்லது சிகிச்சை முறையையோ பாதிக்க மாட்டா என்றும் அறிந்து கொண்டேன்.
2. இந்த ஆராய்ச்சியின் விவரங்களும், அதன் நோக்கங்களும் எனக்குத் தெளிவாக விளக்கப்பட்டன.
3. எனக்கு விளக்கப்பட்ட விஷயங்களைப் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.
4. இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்குபெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியில் இருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்து கொண்டேன்.
5. நோயின் தன்மை பற்றியும், சிகிச்சை முறையின் பக்கவிளைவுகள் பற்றியும் எனக்குத் தெளிவாக எடுத்துரைக்கப்பட்டது.
6. நான் என்னுடைய சுயநினைவுடனும் முழு சுதந்திரத்துடனும் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன்.
7. எனக்கு புற்றுநோய் மருந்து செலுத்துவதற்கும், தேவைப்படும் நேரத்தில் அறுவை சிகிச்சை செய்து கொள்வதற்கும் முழுமனதுடன் சம்மதிக்கிறேன்.
8. எனது நோய் பற்றிய ஆவணங்களைப் பயன்படுத்திக்கொள்ள முழுமனதுடன் சம்மதிக்கிறேன்.

9. இந்த ஆராய்ச்சிக் கட்டுரை வெளியிடப்படும்பொழுது என்னைப் பற்றிய தனிப்பட்ட தகவல்கள் வெளியிடப்பட மாட்டாது என்றும் அறிந்து கொண்டேன்.

கையொப்பம்

PROFORMA

Name: S. No:

Age: IP no:

Address

Complaints	Duration

Clinical examination:

	Right	left
Nipple		
Skin involvement		
Lump size		
Quadrant		
Ulcer		
Chest wall involvement		
Dilated veins		
Mobility		
Skin over the surface		

Axilla:

	Right	left
Number of nodes		
Mobility		
Consistency		
Lymphedema		

Supraclavicular area:

	Right	Left
Number of nodes		
Consistency		
Mobility		

Others:

Cervical nodes:

Respiratory system:

Cardiovascular system:

Hepatomegaly:

Ascites:

Gynec examination:

Nervous system:

Spine:

INVESTIGATIONS

HPE:

PREOP STAGE:

PRECHEMO:

- HEMOGRAM
- RENAL FUNCTION
- LIVER FUNCTION
- ECG
- ECHOCARDIOGRAM

CLINICAL EXAMINATION: (RESPONSE)

	BASELINE	CHEMO I	CHEMO II	CHEMO III	CHEMO IV
TUMOR SIZE					
MOBILITY					
SKIN					
CHEST WALL					
AXILLA					

SONOMAMMOGRAM (RESPONSE):

	BASELINE	CHEMO I	CHEMO II	CHEMO III	CHEMO IV
TUMOR SIZE					
SKIN					
CHEST WALL					
AXILLA					

DOPPLER OF TUMOR (RESPONSE):

	BASELINE	CHEMO I	CHEMO II	CHEMO III	CHEMO IV
PSV					
EDV					
RI					
PI					
FEEDER VESSELS					

POST OPERATIVE DETAILS

PROCEDURE:

POST OP HPE:

- TUMOR SIZE:
- MICROSCOPY:

- NOTTINGHAM INDEX:
- GRADE:
- LYMPHATIC INVASION:
- VASCULAR INVASION:
- MARGINS:
- LYMPH NODES:

- RESPONSE: COMPLETE

  PARTIAL

KEY TO MASTER CHART

CR : Complete Response

ER : Estrogen Receptor

F : Fixed

HPE : Histopathological Examination

I : Involved

IDC : Infiltrating Ductal Carcinoma

L : Left sided

LI : Lymphatic invasion

M : Mobile

N : Negative

NEC : Neuroendocrine Carcinoma

NI : Not Involved

NOS : Not Otherwise Specified

NR : No Response

P : Positive

PgR : Progesterone Receptor

PR : Partial Response

PSV : Peak Systolic Velocity

R : Right Sided

RI : Resistivity Index

VI : Vascular Invasion

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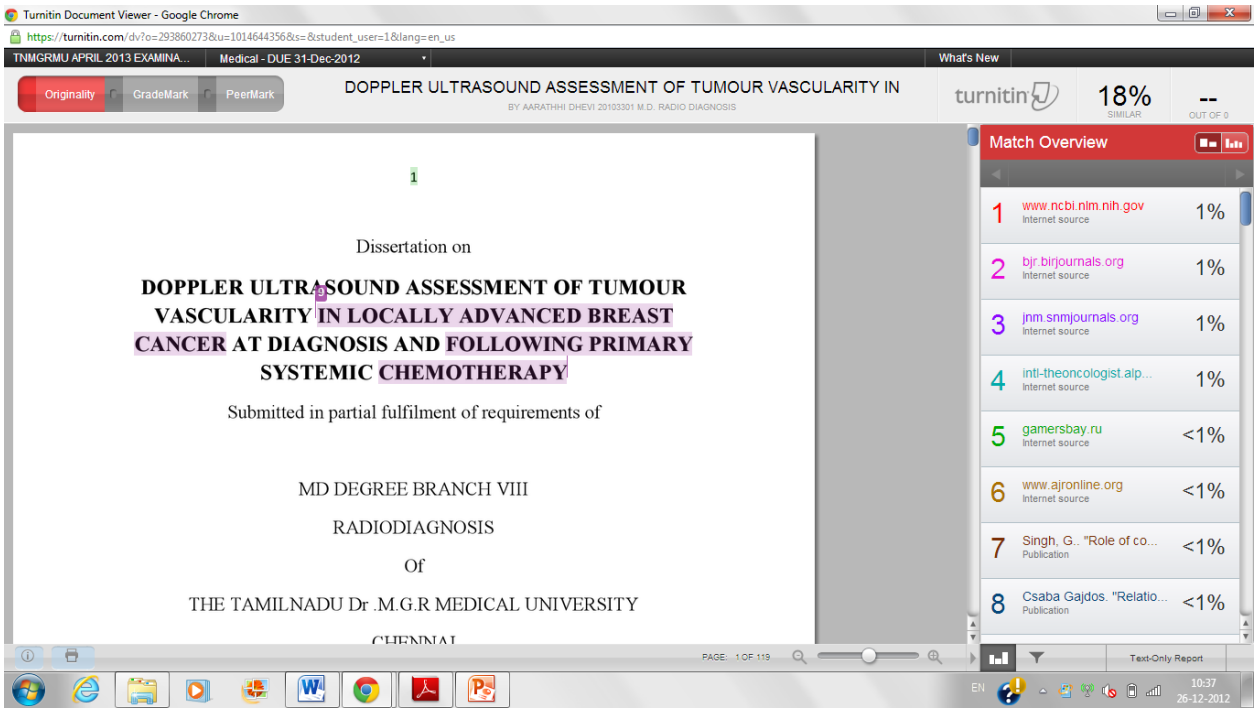
1 Dissertation on DOPPLER ULTRASOUND ASSESSMENT OF TUMOUR VASCULARITY IN LOCALLY ADVANCED BREAST CANCER AT DIAGNOSIS AND FOLLOWING PRIMARY SYSTEMIC CHEMOTHERAPY Submitted in partial fulfilment of requirements of MD DEGREE BRANCH VIII RADIODIAGNOSIS Of THE TAMILNADU Dr .M.G.R MEDICAL UNIVERSITY CHENNAI MADRAS MEDICAL COLLEGE AND RAJIV GANDHI GOVERNMENT GENERAL HOSPITAL CHENNAI 600003 APRIL 2013 2 CERTIFICATE This is to certify that Dr.R.S.AARATHHI DHEVI has been a post graduate student during the period May 2010 to April 2013 at Barnard Institute of Radiology, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai. This Dissertation titled "DOPPLER ULTRASOUND ASSESSMENT OF...



s no	ID	age (yrs)	hpe	type	grade	stage	stage group	clinical examination																														clinical response
								side	size (cm)					skin					chest wall					No of axillary LN					size of nodes (cm)					mobility of nodes					
									0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	
1	157/12	45	IDC	NOS	1	T4BN2M0	IIIB	R	18*17	10*11	9*7	7*6	6*7	I	I	I	I	NI	NI	NI	NI	NI	NI	2	2	1	1	1	3*2	3*2	2*2	1*1	1*1	F	F	M	M	M	PR
2	259/12	57	IDC	NOS	2	T4BN2M0	IIIB	R	12*10	10*12	11*10	10*8	9*8	I	I	I	NI	NI	NI	NI	NI	NI	NI	3	3	2	1	1	2*1	2*1	1*1	1*1	NS	M	M	M	M	M	NR
3	274/12	37	IDC	NOS	2	T4BN3M0	IIIC	L	12*11	10*9	10*10	10*10	9*10	I	I	I	NI	NI	NI	NI	NI	NI	NI	3	1	1	1	1	3*2	3*2	2*2	1*1	1*1	M	M	M	M	M	NR
4	209/12	65	IDC	NOS	2	T4BN1M0	IIIB	R	9*8	6*7	5*5	4*4	4*4	I	I	I	NI	NI	NI	NI	NI	NI	NI	3	2	1	1	1	2*1	2*2	1*1	1*1	<1	M	M	M	M	M	PR
5	172/12	50	IDC	NOS	2	T4BN1M0	IIIB	R	5*4	5*4	5*5	4*3	4*3	I	I	I	NI	NI	NI	NI	NI	NI	NI	2	2	1	1	1	2*2	2*2	<1	<1	<1	M	M	M	M	M	NR
6	339/12	45	IDC	NOS	2	T4BN1M0	IIIB	L	5*4	5*5	4*4	4*3	3*3	I	I	I	NI	NI	NI	NI	NI	NI	NI	1	1	1	1	1	2*2	1*2	2*1	1*1	1*1	F	M	M	M	M	NR
7	429/12	66	IDC	NOS	2	T4BN1M0	IIIB	L	9*8	7*6	5*4	5*4	4*4	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	1	1	1	1	1	2*1	1*1	1*1	<1	<1	M	M	M	M	M	PR
8	255/12	47	IDC	NOS	3	T3N3M0	IIIC	L	8*8	3*4	2*2	0	0	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	1	1	1	1	0	1*1	1*1	<1	<1	<1	M	M	M	M	M	CR
9	426/12	54	IDC	NOS	2	T4BN1M0	IIIB	L	8*6	4*4	0	0	0	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	2	1	1	1	0	3*2	2*2	2*1	1*1	0	M	M	M	M	0	CR
10	418/12	45	IDC	NOS	2	T4BN1M0	IIIB	R	7*6	3*2	1*1	0	0	I	I	NI	NI	NI	NI	NI	NI	NI	NI	1	1	1	0	0	3*2	<1	<1	0	0	M	M	M	0	0	CR
11	352/12	60	IDC	NOS	2	T4BN2M0	IIIB	L	7*5	5*5	5*5	4*4	3*3	I	I	NI	NI	NI	NI	NI	NI	NI	NI	2	2	2	1	1	3*3	3*3	2*2	1*1	<1	F	F	F	M	M	PR
12	378/12	37	IDC	NOS	1	T4BN1M0	IIIB	L	12*10	8*9	8*9	9*10	6*7	I	I	I	NI	NI	NI	NI	NI	NI	NI	1	1	1	1	1	2*1	1*1	1*1	1*1	<1	M	M	M	M	M	PR
13	540/12	60	IDC	NOS	1	T4BN3M0	IIIC	R	6*5	5*5	4*3	3*2	0	I	I	I	NI	NI	NI	NI	NI	NI	NI	2	2	1	1	0	1*1	1*1	1*1	<1	<1	M	M	M	M	M	CR
14	498/12	38	IDC	NOS	2	T3N2M0	IIIA	L	6*6	5*4	0	0	0	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	2	2	2	1	1	2*2	2*2	2*1	2*1	0	F	F	M	M	M	CR
15	781/12	45	IDC	NOS	2	T4CN2M0	IIIB	L	12*8	6*4	4*5	0	0	I	I	I	NI	NI	I	I	I	NI	NI	2	2	1	1	1	2*2	2*2	2*1	1*2	1*1	F	F	M	M	M	CR
16	787/12	57	IDC	NOS	3	T4BN1M0	IIIB	L	8*7	6*6	4*6	4*3	2*1	I	I	NI	NI	NI	NI	NI	NI	NI	NI	3	2	1	1	1	3*2	2*2	1*2	1*1	1*1	M	M	M	M	M	PR
17	619/12	50	IDC	NOS	3	T4BN2M0	IIIB	R	10*8	6*6	5*4	4*4	0	I	I	I	NI	NI	NI	NI	NI	NI	NI	2	1	1	1	1	2*2	2*1	1*1	1*1	<1	M	M	M	M	M	CR
18	698/12	45	IDC	NOS	3	T4BN1M0	IIIB	L	20*15	12*6	6*7	4*5	2*3	I	I	NI	NI	NI	NI	NI	NI	NI	NI	3	2	1	1	1	3*2	2*2	1*1	1*1	<1	M	M	M	M	M	PR
19	569/12	50	IDC	NOS	1	T4BN1M0	IIIB	L	7*6	5*6	5*6	4*3	2*2	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	3	2	1	1	1	3*2	2*2	2*2	<1	<1	M	M	M	M	M	PR
20	710/12	65	IDC	NOS	3	T4BN1M0	IIIB	L	12*9	5*6	3*3	2*1	1*1	I	I	NI	NI	NI	NI	NI	NI	NI	NI	3	3	2	1	0	3*3	3*2	2*1	1*1	0	M	M	M	M	M	PR
21	565/12	65	IDC	NOS	2	T4BN3M0	IIIC	L	5*5	5*5	5*5	4*3	3*2	I	I	NI	NI	NI	NI	NI	NI	NI	NI	2	2	1	1	1	3*2	2*2	2*1	1*1	1*1	M	M	M	M	M	NR
22	695/12	58	IDC	NOS	1	T4BN1M0	IIIB	L	8*6	5*5	2*2	2*1	1*1	I	I	NI	NI	NI	NI	NI	NI	NI	NI	3	2	1	1	1	2*2	1*1	<1	<1	<1	M	M	M	M	M	PR
23	493/12	40	IDC	NOS	1	T4BN1M0	IIIB	L	8*6	5*4	0	0	0	I	I	NI	NI	NI	NI	NI	NI	NI	NI	1	1	1	0	0	2*1	2*1	1*1	0	0	M	M	M	M	M	CR
24	529/12	55	IDC	NOS	2	T4BN1M0	IIIB	L	10*8	5*5	4*3	1*2	0	I	I	NI	NI	NI	NI	NI	NI	NI	NI	2	1	1	1	0	2*2	2*1	1*1	<1	<1	M	M	M	M	M	CR
25	507/12	67	IDC	NOS	2	T4BN3M0	IIIC	L	7*7	4*3	0	0	0	I	I	NI	NI	NI	NI	NI	NI	NI	NI	2	1	1	0	0	2*2	1*1	1*1	0	0	M	M	M	M	M	CR
26	609/12	55	IDC	NOS	3	T4BN2M0	IIIB	R	12*10	6*6	0	0	0	I	I	NI	NI	NI	NI	NI	NI	NI	NI	3	2	1	0	0	1*1	2*1	<1	<1	0	F	M	M	M	M	CR
27	70/12	45	IDC	NOS	1	T4BN1M0	IIIB	R	6*5	4*4	5*3	4*4	3*4	I	I	NI	NI	NI	NI	NI	NI	NI	NI	2	1	1	1	1	2*2	1*1	<1	<1	<1	M	M	M	M	M	PR
28	659/12	27	IDC	NOS	2	T4BN1M0	IIIB	R	10*8	6*4	2*3	0	0	I	I	NI	NI	NI	NI	NI	NI	NI	NI	2	1	1	0	0	2*1	1*1	1*1	<1	<1	M	M	M	M	M	CR
29	760/12	55	IDC	NOS	2	T4BN1M0	IIIB	L	10*10	8*9	9*9	7*5	5*5	I	I	NI	NI	NI	NI	NI	NI	NI	NI	3	1	1	1	1	2*2	1*1	<1	<1	<1	M	M	M	M	M	PR
30	761/12	56	IDC	NOS	2	T4BN0M0	IIIB	R	7*5	5*5	4*3	3*3	3*3	I	I	NI	NI	NI	NI	NI	NI	NI	NI	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	PR
31	657/12	55	IDC	NOS	1	T3N2M0	IIIA	L	8*6	5*4	3*3	4*4	4*3	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	3	2	1	1	1	1*1	2*2	1*1	<1	<1	F	F	M	M	M	PR

s no	ID	age (yrs)	hpe	type	grade	stage	stage group	clinical examination																																clinical response
								side	size (cm)					skin					chest wall					No of axillary LN					size of nodes (cm)					mobility of nodes					
									0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	
32	847/12	55	IDC	NOS	3	T4BN2M0	IIIB	R	12*10	6*7	0	0	0	I	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	3	2	2	2	2	3*2	3*3	2*1	1*1	1*1	F	F	M	M	M	CR
33	854/12	46	IDC	NOS	1	T4BN2M0	IIIB	L	10*10	8*7	7*7	6*6	4*4	I	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	1	1	1	1	0	2*1	2*1	1*1	<1	0	F	M	M	M	M	PR
34	897/12	48	IDC	NOS	2	T4BN1M0	IIIB	L	9*8	0	0	0	0	I	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	1	1	1	1	1	3*2	1*1	<1	<1	0	M	M	M	M	M	CR
35	860/12	50	IDC	NOS	3	T4BN1M0	IIIB	R	5*5	4*5	5*7	8*10	12*8	I	I	I	I	I	NI	NI	NI	NI	NI	3	2	2	2	1	2*2	2*2	3*2	3*2	2*2	M	M	M	M	M	NR
36	898/12	50	IDC	NOS	2	T4BN1M0	IIIB	L	8*8	6*6	0	0	0	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	4	3	1	1	0	2*1	11	<1	<1	<1	M	M	M	M	M	CR
37	899/12	58	IDC	NOS	3	T4BN1M0	IIIB	L	5*5	3*3	1*2	0	0	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	3	2	1	1	0	1*1	1*1	1*1	<1	0	M	M	M	0	0	CR
38	918/12	65	IDC	NOS	2	T3N3M0	IIIC	R	10*8	8*8	6*5	4*3	2*2	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	4	3	1	1	1	2*2	2*1	1*1	1*1	<1	M	M	M	M	M	PR
39	882/12	56	IDC	NOS	2	T4BN3M0	IIIC	R	4*4	4*4	3*2	2*2	2*2	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	3	2	1	1	1	2*1	1*1	1*1	<1	<1	M	M	M	M	M	NR
40	994/12	54	IDC	NOS	3	T3N3M0	IIIC	R	8*8	0	0	0	0	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	1	1	0	0	0	3*2	2*3	<1	0	0	M	M	-	-	-	CR
41	904/12	46	IDC	NOS	2	T4BN1M0	IIIB	R	6*6	0	0	0	0	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	1	0	0	0	0	2*2	1*1	<1	0	0	M	M	M	-	-	CR
42	1101/12	45	IDC	NOS	1	T4BN2M0	IIIB	R	6*4	5*5	5*5	4*5	5*5	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	4	4	2	1	1	3*2	2*2	2*2	2*2	2*2	F	F	M	M	M	NR
43	1090/12	44	IDC	NOS	2	T4BN1M0	IIIB	L	9*8	7*4	5*5	5*4	3*2	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	3	2	2	2	1	2*2	1*2	1*1	<1	<1	M	M	M	M	M	PR
44	926/12	47	IDC	NOS	1	T4BN1M0	IIIB	L	10*8	8*8	7*8	5*5	4*3	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	3	2	2	2	1	2*3	2*1	1*1	<1	<1	M	M	M	M	M	PR
45	947/11	45	IDC	NOS	3	T4BN3M0	IIIC	L	9*9	5*4	3*3	3*2	2*2	I	I	NI	NI	NI	NI	NI	NI	NI	NI	3	2	1	1	1	2*2	1*2	1*1	<1	<1	M	M	M	M	M	PR
46	1159/12	56	IDC	NOS	3	T4BN2M0	IIIB	L	12*8	10*8	8*6	6*6	5*5	I	I	I	NI	NI	NI	NI	NI	NI	NI	3	3	2	1	1	2*3	2*2	1*2	1*1	<1	M	M	M	M	M	PR
47	1157/12	49	IDC	NOS	1	T4BN1M0	IIIB	R	5*4	4*4	0	0	0	I	I	NI	NI	NI	NI	NI	NI	NI	NI	2	2	1	1	1	2*3	2*2	1*1	1*1	<1	M	M	M	M	M	CR
48	1199/12	46	IDC	NOS	1	T4CN1M0	IIIB	R	12*10	8*8	5*5	5*4	3*4	I	I	NI	NI	NI	I	I	NI	NI	NI	3	2	2	2	2	2*2	2*2	1*1	<1	<1	M	M	M	M	M	PR
49	1312/12	45	IDC	NOS	2	T3N3M0	IIIC	R	7*7	5*6	6*6	4*4	3*5	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	2	2	1	1	1	1*1	1*1	<1	<1	<1	M	M	M	M	M	PR
50	1381/12	48	IDC	NEC	1	T4BN1M0	IIIB	L	8*6	4*3	2*3	2*2	2*2	I	I	NI	NI	NI	NI	NI	NI	NI	NI	2	1	1	1	1	2*1	1*1	1*1	<1	<1	M	M	M	M	M	PR
51	1280/12	40	IDC	NOS	1	T4BN2M0	IIIB	L	10*8	9*9	9*8	7*7	7*6	I	I	I	NI	NI	NI	NI	NI	NI	NI	3	1	1	1	1	2*2	2*1	1*1	<1	<1	F	F	M	M	M	NR
52	1422/12	45	IDC	NOS	2	T4BN1M0	IIIB	L	15*10	6*7	5*4	3*4	0	I	I	NI	NI	NI	NI	NI	NI	NI	NI	3	2	1	1	1	2*2	1*1	<1	<1	0	M	M	M	M	M	CR
53	315/12	60	IDC	NOS	2	T3N3M0	IIIC	L	12*10	4*3	0	0	0	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	2	1	1	1	0	1*1	1*1	1*1	<1	0	M	M	M	M	-	CR
54	1112/12	55	IDC	NOS	1	T4BN2M0	IIIB	R	6*5	4*4	2*3	3*3	2*2	I	NI	NI	NI	NI	NI	NI	NI	NI	NI	1	1	1	1	1	1*1	1*1	<1	<1	<1	M	M	M	M	M	PR

s no	ultrasonogram findings										usg response	doppler findings															response	post op HPE					Response	ER	PgR	Her-2/*neu*	stage
	volume (cc)					axilla node size (cm)						PSV (cm/sec)					RI					no of signals						size	LI	VI	SKIN	LN					
	0	1	2	3	4	0	1	2	3	4		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4											
1	236	220	234	226	230	3*3	2*3	2*1	2*2	2*2	NR	10.2	9.8	10.3	9.6	8.9	0.72	0.65	0.5	0.5	0.46	9	7	8	6	7	NR	15*10	Y	Y	I	006/006	NR	N	N	P	T4BN2M0
2	98	100	78	80	80	3*1	2*1	1*1	1*1	<1	NR	23.3	36.3	21.2	7.8	19.9	0.63	0.54	0.55	0.53	0.5	8	9	6	6	6	NR	8*6	Y	Y	NI	003/7	NR	P	P	P	T3N1M0
3	180	175	198	180	190	1*1	2*3	3*3	2*2	1*2	NR	40	49	30	110	78	0.67	0.54	0.55	0.49	0.44	9	9	7	6	7	NR	8*6	Y	Y	I	002/5	NR	N	N	N	T4BN1M0
4	110	90	86	64	52	2*1	<1	0	0	0	PR	28.9	15.9	19.2	94.2	22.3	0.58	0.67	0.74	0.78	0.79	6	5	2	3	2	PR	4*4	N	N	NI	002/2	PR	P	P	N	T2N1M0
5	55	60	58	55	55	2*2	2*2	<1	<1	<1	NR	56.8	19.2	15.8	26.3	22.9	0.6	0.58	0.62	0.42	0.4	6	5	6	7	7	NR	5*4	Y	Y	NI	002/7	NR	P	P	P	T2N1M0
6	60	56	54	50	50	2*2	2*2	2*1	2*1	1*1	NR	54.3	64.7	54.2	49.8	9.85	0.71	0.66	0.62	0.56	0.5	9	8	8	6	6	NR	4*4	Y	Y	NI	003/10	NR	N	P	P	T2N1M0
7	54	56	40	34	32	2*1	1*1	<1	<1	<1	NR	42.8	44.8	23.2	28.4	30.1	0.7	0.76	0.8	0.8	0.8	9	6	3	3	1	PR	2*1	Y	Y	NI	001/7	PR	P	P	N	T1N1M0
8	96	56	34	24	20	1*1	1*1	1*1	<1	<1	PR	23.1	19.3	16.7	12.9	16.8	0.56	0.76	0.78	0.82	0.88	11	6	2	1	1	PR	2*2	Y	Y	NI	005/8	PR	P	P	N	T1N2M0
9	120	70	40	15	15	1*1	1*1	1*1	0	0	PR	20.9	27.1	15.8	29.1	38.6	0.68	0.84	0.86	0.86	0.9	4	4	4	5	1	PR	5*4	Y	Y	NI	001/3	NR	N	N	N	T1N1M0
10	98	36	24	0	0	3*2	2*2	1*1	0	0	CR	47.2	39.8	15.8	20.6	0	0.68	0.86	0.86	0.92	0	5	4	4	2	1	CR	0	-	-	-	0/7	CR	N	N	P	T0N0M0
11	100	90	90	65	50	2*2	3*1	2*1	1*1	<1	PR	32.5	35.7	13.2	12	8.8	0.54	0.68	0.7	0.74	0.88	8	3	2	2	1	PR	2*1	Y	Y	NI	001/6	PR	P	P	N	T1N1M0
12	178	134	126	148	98	3*2	3*2	3*1	2*2	2*2	NR	15	15.3	30.3	42.8	15.9	0.84	0.79	0.55	0.62	0.78	3	1	2	4	1	PR	4*3	Y	Y	NI	005/9	PR	P	P	N	T2N2M0
13	110	90	45	30	25	1*1	1*1	<1	<1	<1	PR	34.7	15.8	19.2	0	0	0.72	0.76	0.76	0	0	4	4	3	0	0	CR	0	-	-	-	0/9	CR	N	N	N	T0N0M0
14	98	76	34	20	20	2*2	2*1	1*1	1*1	1*1	PR	15.8	28.6	29.8	22.7	13.8	0.54	0.7	0.76	0.8	0.8	8	4	2	1	1	PR	0	-	-	-	0/8	CR	N	P	N	T0N0M0
15	198	98	56	42	30	1*1	1*1	1*1	1*1	<1	PR	35.7	36.2	19.3	19.2	15.7	0.58	0.72	0.8	0.85	0.91	10	6	6	3	1	PR	2*2	Y	Y	NI	005/7	PR	P	P	P	T1N2M0
16	110	90	65	30	28	3*2	1*1	1*1	<1	<1	PR	15.9	32.2	42.1	15.8	19.3	0.4	0.6	0.62	0.62	0.68	10	9	6	2	2	PR	2*1	Y	Y	NI	0/0	PR	N	N	N	T1N0M0
17	190	58	46	30	25	3*1	3*2	3*1	1*1	<1	PR	63.7	206	55.8	44.9	12.8	0.3	0.58	0.6	0.64	0.7	10	9	6	3	1	PR	3*3	Y	Y	NI	001/5	PR	N	N	N	T2N1M0
18	240	198	96	54	30	3*2	2*2	2*1	1*1	<1	PR	34.7	15.8	19.2	15.4	23.8	0.43	0.6	0.68	0.78	0.86	10	10	9	5	2	CR	2*2	Y	Y	NI	0/5	PR	P	P	N	T1N0M0
19	90	54	42	36	24	1*1	1*2	2*1	1*1	<1	PR	28.1	15.8	10.9	49.2	23.9	0.74	0.74	0.77	0.82	0.88	2	3	1	1	1	PR	2*2	Y	Y	NI	002/6	PR	P	P	P	T1N1M0
20	198	110	56	45	22	2*2	2*2	1*1	1*1	<1	PR	25.4	44.4	7.7	20.4	0	0.45	0.7	0.72	0.78	0	6	5	3	2	0	CR	0	-	-	NI	0/9	CR	P	P	N	T0N0M0
21	76	54	34	23	20	3*2	2*2	1*1	1*1	1*1	PR	27.3	27.1	26.1	32.1	44.8	0.5	0.53	0.5	0.58	0.6	5	4	3	4	1	PR	2*2	Y	Y	NI	001/10	PR	N	N	N	T1N1M0
22	108	76	45	30	20	2*2	2*2	1*1	1*1	<1	PR	32.5	35.7	13.2	12	8.8	0.56	0.78	0.82	0.88	0.9	10	8	6	4	2	PR	2*2	Y	Y	NI	002/10	PR	N	P	N	T2N1M0
23	122	76	34	25	23	1*1	1*1	<1	<1	<1	PR	66.9	20.9	101.8	54.9	80.2	0.59	0.72	0.76	0.78	0.86	5	4	6	2	1	PR	1*1	Y	Y	NI	001/8	PR	P	P	N	T1N1M0
24	165	78	44	32	22	2*1	1*1	1*1	<1	<1	PR	28.1	15.8	0	0	0	0.6	0.78	0	0	0	7	6	0	0	0	CR	0	-	-	NI	0/12	CR	N	N	N	T0N0M0
25	86	53	22	0	0	2*1	1*0.5	.5*0.	0	0	CR	28.5	28.3	65.3	73.4	0	0.66	0.85	0.91	0.89	0	3	3	3	1	0	CR	0	-	-	NI	0/9	CR	N	P	P	T0N0M0
26	122	68	42	12	0	2*2	1*1	<1	0	0	CR	31.1	22.7	26.9	11.9	8.3	0.44	0.68	0.7	0.68	0.78	11	5	1	1	1	PR	1*1	Y	Y	NI	001/10	PR	N	P	N	T1N1M0
27	56	40	38	42	36	2*3	1*1	1*2	1*1	<1	NR	54.9	23.9	12.8	21.9	23.8	0.76	0.74	0.68	0.68	0.6	3	1	1	1	1	NR	4*4	Y	Y	NI	0/9	NR	N	N	P	T2N0M0
28	76	54	32	25	20	1*1	1*1	1*1	<1	<1	PR	15.9	32.2	42.1	15.8	19.3	0.45	0.62	0.75	0.8	0.82	6	3	1	1	1	PR	2*2	-	-	NI	0/14	PR	N	N	N	T1N0M0
29	104	98	76	78	68	1*1	2*1	1*1	<1	<1	NR	65.9	42.8	21.8	16.9	9.3	0.5	0.52	0.68	0.7	0.67	5	2	1	1	1	PR	2*3	Y	Y	NI	002/9	PR	N	P	P	T2N1M0
30	68	54	45	40	34	0	0	0	0	0	PR	33.1	24.2	69.3	28.2	32.6	0.54	0.58	0.67	0.64	0.62	3	2	1	1	1	PR	3*2	Y	Y	NI	0/8	PR	P	P	N	T2N0M0
31	78	56	34	32	28	2*1	1*1	1*1	1*1	<1	PR	23.1	66.1	34.8	12.9	9.1	0.78	0.82	0.76	0.8	0.72	6	2	1	1	1	PR	4*3	Y	Y	NI	0/8	PR	N	N	N	T2N0M0

s no	ultrasonogram findings										usg response	doppler findings															response	post op HPE					Response	ER	PgR	Her-2/*neu*	stage
	volume (cc)					axilla node size (cm)						PSV (cm/sec)					RI					no of signals						size	LI	VI	SKIN	LN					
	0	1	2	3	4	0	1	2	3	4		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4											
32	148	79	56	44	34	2*2	2*2	1*1	<1	<1	PR	18.9	28.2	25.7	0	0	0.38	0.57	0.6	0	0	11	4	2	0	0	CR	0	-	-	NI	0/9	CR	N	N	P	T0N0M0
33	100	86	64	50	34	3*1	2*1	1*1	<1	<1	PR	57.5	22.9	30.1	44.2	34.8	0.86	0.86	0.75	0.78	0.8	8	6	6	4	4	PR	4*4	Y	Y	NI	001/8	PR	N	P	N	T3N1M0
34	78	34	20	0	0	1*1	<1	<1	<1	<1	CR	28.9	15.9	17.9	22.9	44.6	0.56	0.72	0.68	0.7	0.78	8	6	3	1	1	PR	0	-	-	-	0/8	CR	N	P	P	T0N0M0
35	45	40	76	84	90	1*1	1*1	2*1	1*1	1*1	NR	51.3	53.9	68.3	9.8	20.8	0.92	0.88	0.82	0.7	0.64	4	3	2	2	2	NR	11*9	Y	Y	I	004/13	NR	P	P	P	T4BN2M0
36	66	54	34	24	24	<1	<1	<1	<1	<1	PR	31.5	26.8	19.2	21.8	12.8	0.68	0.76	0.78	0.78	0.8	4	3	1	1	1	PR	2*3	Y	Y	NI	002/5	PR	N	P	N	T2N1M0
37	56	43	24	20	0	2*2	2*1	1*1	1*1	<1	CR	42.1	23.6	0	0	0	0.46	0	0	0	0	2	1	0	0	0	CR	2*3	Y	Y	NI	002/5	PR	P	P	P	T2N1M0
38	98	66	45	30	24	2*1	1*1	1*1	<1	<1	PR	23.4	18	16.7	17	23	0.55	0.7	0.75	0.78	0.8	7	3	2	1	1	PR	3*2	Y	Y	NI	004/6	PR	N	N	N	T2N2M0
39	56	50	46	26	20	2*1	1*1	1*1	<1	<1	PR	34.5	24.7	21	19.8	19	0.52	0.68	0.79	0.79	0.82	9	4	2	1	1	PR	2*2	Y	Y	NI	002/7	PR	N	P	N	T2N1M0
40	76	21	0	0	0	1*1	1*1	<1	0	0	CR	17.5	39.9	15.6	32.1	23	0.43	0.58	0.6	0.62	0.72	5	1	1	1	1	PR	1*1	N	N	NI	0/6	PR	P	P	N	T1N0M0
41	56	34	12	0	0	2*1	1*1	<1	0	0	CR	17	18.4	13	9.8	11.1	0.6	0.76	0.78	0.8	0.8	4	4	1	1	1	PR	1*1	N	N	NI	001/5	PR	P	P	N	T1N0M0
42	74	70	68	70	52	1*1	1*1	<1	<1	<1	NR	14.3	14.1	6.3	5.8	4.9	0.81	0.67	0.65	0.56	0.52	5	3	2	3	4	NR	3*2	Y	Y	NI	004/12	PR	N	P	N	T2N2M0
43	98	86	74	70	46	3*2	2*2	2*1	<1	<1	PR	10.8	12.7	9.8	12.1	7.6	0.63	0.7	0.68	0.76	0.8	6	3	2	1	1	PR	2*2	Y	Y	NI	001/11	PR	N	N	N	T1N1M0
44	102	78	56	42	32	2*2	1*2	1*1	<1	<1	PR	34.1	36.6	29.6	24.3	19.1	0.92	0.88	0.85	0.8	0.76	7	3	3	2	3	PR	3*3	Y	Y	NI	002/8	PR	P	N	N	T2N1M0
45	122	78	46	34	24	1*1	1*1	<1	<1	<1	PR	34.1	58.4	0	0	0	0.56	0.6	0	0	0	8	4	1	1	1	CR	1*1	Y	Y	NI	004/9	PR	N	N	P	T1N2M0
46	132	100	56	44	32	3*1	3*1	2*2	1*1	<1	PR	10.1	8.9	11.2	15.8	22.9	0.48	0.63	0.68	0.78	0.82	9	3	2	2	1	PR	5*4	Y	Y	NI	002/9	PR	P	N	N	T2N1M0
47	56	45	32	20	0	2*2	1*2	<1	0	0	CR	8.9	11.1	12.8	21	9.8	0.66	0.87	0.9	0.91	0.9	8	3	2	1	1	PR	3*1	N	N	NI	0/9	NR	P	P	N	T2N0M0
48	132	86	76	46	24	1*1	2*1	2*2	<1	<1	PR	36.5	22.5	19.8	23.6	21.1	0.7	0.74	0.8	0.82	0.78	9	3	2	2	1	PR	6*3	Y	Y	NI	007/8	PR	P	P	N	T3N3M0
49	76	54	50	46	34	2*2	2*1	1*1	<1	<1	PR	24.4	18.9	14.8	14	12.8	0.57	0.64	0.78	0.8	0.82	7	4	2	1	1	PR	4*3	Y	Y	NI	004/9	PR	N	P	N	T2N2M0
50	76	56	38	24	24	2*1	3*2	2*1	1*1	1*1	PR	16.8	21.8	19.7	15.9	20	0.52	0.7	0.76	0.75	0.74	8	3	1	1	1	PR	1*1	N	N	NI	002/8	PR	P	P	N	T1N1M0
51	102	88	76	68	70	2*1	1*1	1*1	1*1	<1	NR	21.6	18.7	17.6	14.4	9.7	0.9	0.86	0.79	0.82	0.82	9	4	3	1	1	PR	3*2	N	N	NI	001/9	PR	N	N	N	T2N1M0
52	142	196	46	34	10	1*1	1*1	<1	0	0	PR	87.5	23.6	43.1	12.7	13.8	0.58	0.76	0.78	0.92	0.9	10	3	1	1	1	PR	1*1	Y	Y	NI	005/11	PR	P	P	N	T1N2M0
53	112	78	46	24	26	1*1	1*1	<1	<1	<1	PR	57.8	43.8	23	12.1	9.7	0.66	0.82	0.8	0.88	0.9	6	5	2	1	1	PR	2*1	Y	Y	NI	006/7	PR	P	P	N	T1N2M0
54	56	36	30	28	25	2*1	1*1	1*1	1*1	1*1	PR	23.1	19.2	13.8	17.2	11.4	0.67	0.78	0.78	0.8	0.86	8	3	2	1	1	PR	3*2	Y	Y	NI	001/11	PR	N	P	N	T2N1M0